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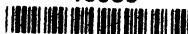
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# SERIAL CORRELATION IN THE ANALYSIS OF TIME SERIES<sup>1</sup>

RICHARD L. ANDERSON

From the Department of Mathematics, Iowa State College

Received June 10, 1941

The problem of serial correlation was first introduced by Yule (8), who stated that the standard error test of significance was invalidated if our observations were not independent of one another. Yule adopted the term *lagged serial correlation* to denote the relationship between successive values of some variable  $x$ . If we have a set of  $n$  ordered observations,  $x_1, \dots, x_n$ , with mean 0, we will define the serial correlation coefficient for lag  $L$  to be

$$R_L = \frac{C_L}{V} = \frac{x_1x_{L+1} + x_2x_{L+2} + \dots + x_Nx_L}{\sum x_i^2}.$$

The writer was first introduced to this problem in a recent study of the harmonic components of the cyclical deviations of agricultural prices (1). The ordinary tests of significance of the harmonic terms derived by Walker (2) and Fisher (4) could not be used directly. The most obvious difficulty was the determination of the proper number of degrees of freedom in the price series. A tentative solution suggested by E. B. Wilson was adopted at that time (6). Wilson proposed that a sequence of the serial correlation coefficients,  $R_1, R_2, \dots$ , be computed to determine the first zero  $R_L$ . The best estimate of the true number of degrees of freedom was taken to be the number of items in the series divided by  $L$ .

The concept of serial correlation was extended by H. Wold (7), who proposed that a graph of the various serial correlation coefficients, called the correlogram, be used to determine whether the method of hidden periodicities, linear auto-regression, or moving averages should be used to analyze a time series. If the correlogram consists of a more or less regular sequence of harmonic terms with no damping of the amplitudes, the method of hidden periodicities should be used. If all amplitudes become almost zero (not zero because of sampling variation) after a certain lag, the method of moving averages is appropriate. If these amplitudes are merely damped, the method of linear auto-regression can be used.

All these applications of serial correlation depend upon a table of significance levels of  $R_L$ , which has never been given before. We have worked out the distributions of the serial correlation coefficient for arbitrary lags. The distributions included in this thesis have been based on

<sup>1</sup>Original thesis submitted June, 1941. Doctoral thesis number 611.

the assumption that the  $x$ 's have a zero mean, but in future research we intend to derive the distributions and significance levels for  $R_L$  corrected for a sample mean and perhaps any trend. The distribution of the serial covariance  $C_L$  has been indicated, but it is of little utility for most practical purposes; hence, only the distributions of  $R_L$  will be discussed in this abstract.

For lag 1 and large  $N$ , we found that  $y = NR_1/(1+2R_1^2)$  is normally distributed with variance 1 and mean 0. The significance levels for  $R_1$  can be found by substituting the significance levels of  $y$  in the formula  $R_1 = y/\sqrt{N-2y^2}$ .

In the determination of the distribution of  $R_1$  for small samples, a result given by W. G. Cochran on quadratic forms was utilized (3). For our problem, it was found that  $C_1$ , the covariance for lag 1, is dis-

distributed as  $\sum_{i=1}^N \lambda_i u_i$ .  $u_i$  is distributed as  $\chi^2$  with 1 degree of freedom, and the  $\lambda$ 's are the roots of the characteristic equation of the coefficients of the  $x$ 's in  $C_1$ . This equation takes the form

$$F_{1,N}(\lambda) = \prod_{i=1}^N (-\lambda_i + \cos \frac{2\pi i}{N}) = 0,$$

so that  $\lambda_i = \cos \frac{2\pi i}{N}$ . At the same time,  $V$  is distributed as  $\sum_{i=1}^N u_i$ .

Using these distributions of  $C_1$  and  $V$ , we determined the following general probability function for  $R_1$  and  $N$  odd:

$$P(R_1 > R') = 1 - \sum_{i=m}^{\frac{N-1}{2}} (R' - \lambda_i)^{-\frac{N-2}{2}} / a_i \text{ for } \lambda_m \leq R' \leq \lambda_{m+1},$$

where  $a_i = \prod_{j=1}^{N-1} (\lambda_j - \lambda_i) \sqrt{1 - \lambda_i}$ ,  $j \neq i$ . No general formula was found

for  $N$  even; the significance levels of  $R_1$  for even  $N > 6$  have been determined by interpolating between the levels for the adjacent odd  $N$ .

For a general lag  $L$ , certain generalizations concerning the characteristic equations  $F_{L,N} = 0$  may be set down: (1) If  $L$  and  $N$  have no common factor,  $F_{L,N} = F_{1,N}$ . (2) If  $L$  and  $N$  have a common factor  $a$ ,  $F_{L,N} = (F_{1,N}/a)^a$ . The second statement indicates that if  $L$  is a multiple of  $N$ , so that  $N = pL$ ,  $F_{L,N} = (F_{1,p})^L$ . Only the distributions of  $R_L$  for which  $L$  is a factor of  $N$  have been derived. This lag has been called the primary lag. The distributions of any of the other  $R_L$ 's will be included in this group. As an example, for  $N = 14$ ,  $R_4$ ,  $R_6$ ,  $R_8$ ,  $R_{10}$ , and  $R_{12}$  are all distributed like  $R_2$  (lag 2 is the primary lag).

For  $p = 2$  ( $N = 2L$ ), the general probability function is

$$P(R_L > R') = \frac{1}{\beta(L/2, L/2)} \int_{y=0}^{\frac{1}{2}(1-R')} y^{L/2-1} (1-y)^{L/2-1} dy.$$

Pearson has tabulated these incomplete Beta functions (5). In his notation,  $P(R_L > R') = I_x(L/2, L/2)$ , where  $x = (1-R')/2$ . For  $p = 3$ , the probability function is  $P(R_L > R') = I_x(L/2)$ , where  $x = 2(1-R')/3$ . For  $p = 4$ , the density function of  $R_L$  is

$$D(R_L) = k_L \int_{x=0}^{1-R_L} x^{L-1} X^{L/2-1} dx,$$

where  $k_L = \frac{\Gamma(2L)}{2^{L-1} \Gamma^2(L/2) \Gamma(L)}$  and  $X = [x^2 - 2x + (1 - R_L^2)]$ . This

integral was rather easily evaluated for  $L$  even by expanding the integrand in a series of the form  $\sum x^m X^n$ . We have interpolated for most of the significance levels for  $L$  odd.

For  $p > 4$ , the distributions have been solved for lag 2 but only for  $p = 5, 6, 7, 8$ , and 9. Only the integrands have been set up for other lags.

The 1 per cent and 5 per cent significance levels for  $R_1$  have been tabulated for  $N$  up to 45; for general  $R_L$ , those significance levels for  $p = 2$  and 3 have been extended for lags up to 50 and the levels for  $p = 4$  up to lag 16.

No significance levels have been tabulated for  $R_L$  corrected for the sample mean, but most of the probability formulas have been included. The large sample distribution is the same as before except that  $N$  is replaced by  $N - 1$ . The probability function for  $N$  odd is  $P(R_1 > R') =$

$$1 - \sum_{i=m}^{k+1} (R' - \lambda_i)^k / \alpha_i, \text{ for } \lambda_m \leq R' \leq \lambda_{m-1}, \text{ where } k = (N-3)/2, \alpha_i =$$

$$\prod_{j=1}^{k+1} (\lambda_j - \lambda_i) \text{ for } j \neq i, \text{ and } m \geq 2. \text{ Similarly for } N \text{ even, } P(R_1 > R') =$$

$$\sum_{i=1}^m (\lambda_i - R')^k / \alpha_i, \text{ for } \lambda_{m+1} \leq R' \leq \lambda_m, \text{ where } \alpha_i = \prod_{j=1}^2 (\lambda_j - \lambda_i) \sqrt{1 + \lambda_i} \text{ for}$$

$j \neq i$ . For  $p = 2$ ,  $P(R_L > R') = I_x[L/2, (L-1)/2]$ , where  $x = (1 - R'/2)$ ; for  $p = 3$ ,  $P(R_L > R') = I_x[L, (L-1)/2]$ , where  $x = 2(1 - R')/3$ .

A few examples taken from the data used in my recent study of agricultural prices (1) have been included to demonstrate how to calculate the serial correlation coefficients and how to use the results in interpreting the data.

In conclusion, it should be noted that this manuscript is merely an introduction to the problem of serial correlation and that we intend extending the results to include data corrected for trend as well as higher lags.

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# RELATIVE REACTIVITIES OF SOME ORGANOMETALLIC COMPOUNDS<sup>1</sup>

LESTER D. APPERSON

From the Department of Chemistry, Iowa State College

Received May 22, 1941

## A. ORGANOALUMINUM HALIDES

Organoaluminum halides have been found to possess an order of reactivity which is comparable to simple organoaluminum compounds. There is not much difference in reactivity between  $R_2AlX$  compounds and  $RAI_2$  compounds. Likewise, alkyl- and arylaluminum halides exhibit the same order of reactivities.

All types of organoaluminum halides give positive color tests when they are warmed for 5 minutes with Michler's ketone. Benzonitrile and the mixed phenylaluminum iodides produce a 2 per cent yield of benzophenone. Acetyl chloride and *p*-tolylaluminum dichloride give a 75 per cent yield of methyl *p*-tolyl ketone in xylene solution. Propiophenone is obtained in a 95 per cent yield from benzoyl iodide and the mixed ethylaluminum iodides in benzene solution. Benzoyl iodide and the mixed phenylaluminum iodides produce a 33 per cent yield of benzophenone in petroleum ether (b.p., 90-115°) solution. In general, benzoyl iodide is more reactive than benzoyl chloride toward organoaluminum halides. Reactions with benzoyl chloride are summarized in Table 1.

The proper choice of solvent has considerable effect on the yield of product. Aromatic hydrocarbon solvents give the best results. The yield of propiophenone from benzoyl chloride and the mixed ethylaluminum iodides is practically negligible when diethyl ether is used as the reaction medium. Acid anhydrides react quite readily with organoaluminum halides to give ketones, but only one carbonyl group is involved in the reaction. Acetic anhydride and the mixed ethylaluminum chlorides produce a 40 per cent yield of methyl ethyl ketone in benzene solution. The yield of propiophenone from benzoic anhydride and the mixed ethylaluminum iodides is 45 per cent in benzene solution. A 45 per cent yield of benzoic acid is also obtained.

Ketones are condensed by organoaluminum halides, and for that reason previous workers have concluded that Friedel-Crafts ketone syntheses cannot involve the intermediate formation of organoaluminum halides.<sup>2</sup> However, in Friedel-Crafts reactions the ketones are always combined with aluminum chloride in complexes, and it is necessary to hydrolyze the complexes to obtain the free ketones. It has been found that ketone-aluminum chloride complexes are unaffected by organo-

<sup>1</sup>Original thesis submitted July 16, 1940. Doctoral thesis number 588.

<sup>2</sup>Leone and Braicovic, *Gazz. chim. ital.*, 55, 301 (1925).

TABLE 1  
REACTIONS OF BENZOYL CHLORIDE AND ORGANOALUMINUM HALIDES

Organanoaluminum Halide	Solvent	Product	Percentage Yield
$[(C_6H_5)_2AlI + C_6H_5AlCl]$	benzene	propiophenone	90
$[(C_6H_5)_2AlI + C_6H_5AlCl]$	carbon disulfide	propiophenone	85
$[(C_6H_5)_2AlI + C_6H_5AlCl]$	tetrachloroethane	propiophenone	54
$[(C_6H_5)_2AlI + C_6H_5AlCl]$	petroleum ether	propiophenone	48
$[(C_6H_5)_2AlI + C_6H_5AlCl]$	xylene	benzophenone	47.5
$[(C_6H_5)_2AlI + C_6H_5AlCl]$	petroleum ether	benzophenone	12
$p\text{-CH}_2C_6H_4AlCl_2$	xylene	phenyl <i>p</i> -tolyl ketone	60
$p\text{-CH}_2C_6H_4AlCl_2$	petroleum ether	phenyl <i>p</i> -tolyl ketone	30
$(C_6H_5)_2AlCl$	benzene	propiophenone	67

aluminum halides under moderate reaction conditions and that the ketones can be recovered upon hydrolysis. Furthermore, acid chloride-aluminum chloride complexes react quite readily with organoaluminum halides to give good yields of ketones. Therefore, the intermediate formation of organoaluminum halides as a mechanism for Friedel-Crafts ketone syntheses is justified on the basis of the reactivity of the organoaluminum compounds. It is, however, still a matter of speculation whether intermediate organoaluminum compound formation is the true mechanism, since it has never been demonstrated that aluminum chloride and aromatic hydrocarbons will form organoaluminum halides.

The organoaluminum halides have been prepared by standard procedures with slight modifications in a few preparations. New organoaluminum halides used in these studies include the mono-etherate of diethylaluminum chloride (b.p., 100-102°/3 mm.), the mixed *n*-butyl-aluminum iodide etherates (b.p., 100-170°/5 mm.), the mixed *n*-butyl-aluminum chloride etherates (b.p., 100-170°/25 mm.) and *p*-tolylaluminum dichloride.

### B. DIVALENT ORGANOLEAD RADICALS

The studies on divalent organolead radicals were undertaken with the dual purpose of devising improved methods for their preparation and determining their reactivity in comparison with trivalent and tetravalent organolead compounds.

Red solutions are obtained when reactions between Grignard reagents and lead chloride are carried out at -2 to 0°, but no divalent organolead radicals could be isolated by the writer.

Attempts to prepare diphenyllead by the reduction of diphenyllead dihalides with alkali metals in liquid ammonia solution have been unsuccessful. The expected reaction,  $(C_6H_5)_2PbX_2 + 2M \rightarrow (C_6H_5)_2Pb + 2MX$ , either does not take place or more likely occurs and the diphenyllead reacts further to give triphenyllead and inorganic lead compounds. The addition of two equivalents of alkali metals to one equivalent of the diphenyllead dihalides in liquid ammonia produces black-colored solu-

tions which yield triphenyllead and inorganic lead compounds upon evaporation of the liquid ammonia. Addition of ethyl bromide to the black solution gives a compound which is probably triphenyllead with ethyl bromide of crystallization.

The addition of four equivalents of lithium to diphenyllead dihalides in liquid ammonia produces black-colored solutions which give triphenyllead and inorganic lead compounds upon evaporation of the liquid ammonia. Treatment of the black solution with ethyl bromide and benzyl chloride gives triphenylethyllead and triphenylbenzyllead, respectively. The reverse addition of one equivalent of diphenyllead dihalides to four equivalents of lithium dissolved in liquid ammonia gives red solutions which yield chiefly inorganic lead compounds and a small amount of triphenyllead upon evaporation of the solvent. Addition of ethyl bromide to the red solution gives inorganic lead compounds and an oil which is about 60 per cent diphenyldiethyllead. The remainder of the oil is triphenylethyllead (25 per cent), phenyltriethyllead (8 per cent) and tetraethyllead (4 per cent). The main reaction is probably the formation of dilithium diphenyllead, hitherto unknown, which reacts with ethyl bromide to give diphenyldiethyllead. The previously reported constants ( $n_D^{18^\circ}$ , 1.5939;  $d^{20^\circ}$ , 1.6435) for diphenyldiethyllead could not be checked<sup>4</sup>. A sample of diphenyldiethyllead, prepared from diphenyllead dibromide and ethylmagnesium bromide, gives the following data:  $n_D^{20^\circ}$ , 1.6128;  $d^{20^\circ}$ , 1.6410; b.p., 160-162°/3 mm. A more carefully fractionated sample, obtained from liquid ammonia reactions, gives very similar constants ( $n_D^{20^\circ}$ , 1.6150;  $d^{20^\circ}$ , 1.6401; b.p., 158-161°/2 mm.).

Lithium and sodium are the best metals to use in the preparation of  $M_2PbR_2$  compounds, and the dibromide and dichloride are the best diphenyllead dihalides to use in the preparation of dilithium diphenyllead. Attempts to isolate dilithium diphenyllead from the red liquid ammonia solutions give only decomposition products. The addition of diphenyllead dibromide to dilithium diphenyllead yields only triphenyllead and inorganic lead compounds. The same products are obtained by the addition of ammonium bromide to dilithium diphenyllead.

Miscellaneous attempted preparations of diphenyllead include the reduction of the diphenyllead dihalides by catalytic hydrogenation, hydrazine hydrate, and aluminum metal. The latter two reagents do not affect a reduction under the mild reaction conditions, but catalytic hydrogenation at 30 pounds pressure completely reduces the diphenyllead dihalides to benzene and inorganic lead compounds. Diphenyllead di-iodide gives biphenyl instead of benzene.

The reaction of lead mercaptide with phenyllithium probably involves the intermediate formation of diphenyllead, but only triphenyllead, tetraphenyllead, and metallic lead are isolated.

<sup>4</sup> Möller and Pfeiffer, Ber., 49, 2443 (1916).

# THE PHYSIOLOGIC AND ECONOMIC EFFICIENCY OF RATIONS CONTAINING DIFFERENT AMOUNTS OF GRAIN WHEN FED TO DAIRY CATTLE<sup>1</sup>

KENNETH MAXWELL AUTREY

*From the Laboratories of Dairy Husbandry and Animal Nutrition, Department of  
Animal Husbandry, Iowa State College*

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In 1938-39 a dairy cattle feeding experiment was conducted at this station to compare a ration of roughage alone with rations of roughage and grain. Fifteen Holstein cows of the station herd were used in this trial. They were divided into five trios, the animals of each trio being similar in age, size, stage of lactation, and production. The fifteen cows were then randomly divided into three groups of five cows each, each animal of a trio being placed in a group different from the corresponding two. The experiment consisted of three experimental periods of seven weeks each and was of the double switchback type. Group I received a ration of roughage alone in the first period, while Group II received roughage plus a limited amount of grain (1 pound for each 8 pounds of milk produced). The rations for these two groups were reversed at the end of Period I, and then they were returned to their original respective rations for the third period. Group III served as a check and received a ration of roughage plus a full amount of grain (1 pound for each 4 pounds of milk produced) throughout the experiment.

One of the cows was removed from the experiment as a T.B. reactor in the second period, and in order to keep the groups uniform, the corresponding two animals in the other groups were also removed.

A summary of the results shows that the consumption of dry matter was considerably greater by the animals receiving grain than those receiving only roughage feeds. Although the cattle on roughage alone lost no weight, they seemed less thrifty than those in opposing groups, and there was a greater incidence of animals going off-feed when they received no grain. Milk production by cows on limited grain was considerably greater than that on roughage alone, and likewise greater on full grain than limited grain feeding. The difference in both cases were significant. The returns above feed cost, using Iowa prices of 1938-39, favored full grain feeding over limited grain, and the latter over roughage alone.

The results of this experiment were not conclusive and the design was not appropriate for an adequate statistical interpretation of the data. Hence a second experiment was conducted in 1939-40 to further

<sup>1</sup>Original thesis submitted June, 1941. Doctoral thesis number 608.

study the physiologic and economic efficiency of rations containing different amounts of grain when fed to dairy cattle.

Eighteen Holstein cows were used in this investigation. They were divided into six outcome groups of three cows each, the animals in each trio being selected for as much uniformity as possible, in age, size, stage of lactation, and production.

The experiment was confined to one lactation, and was of a modified change-over type. It consisted of three six-week experimental periods with one week between the periods for changing rations. The plan was such that each cow received a different ration during each period; and each ration was preceded by each of the other rations an equal number of times throughout the trial, when all cows were considered. The rations were allotted in such a way that each animal of a group received one different from that of the other two individuals of the trio during a given period. Although this method of allotment is not entirely random, it was done so that any carry-over effects on milk production or nutrient consumption which might occur in changing from one ration to another could be properly evaluated in the analysis of the results. This plan also permits tests of significance of the differences between the ration effects.

The rations tested in this experiment were: (A) alfalfa hay and corn silage; (B) alfalfa hay, corn silage, and grain fed at the rate of 1 pound to each 7 pounds of milk produced; and (C) alfalfa hay, corn silage, and grain fed at the rate of 1 pound to each 3.5 pounds of milk produced.

The constituents of the grain mixture, in parts by weight, were: Corn and cob meal, 4; oats, 4; cracked soybeans, 1; bone meal, 0.25; and salt, 0.15. All animals, while receiving roughage alone, were fed a mineral supplement consisting of bone meal and salt. The cows were fed all the hay and silage they would eat, and had access to water at all times.

For certain of the analyses given in the results, namely, calculation of the measures of physiologic and economic efficiency, the data of the first experiment were combined with those of the latter one.

The results show that the total dry matter consumption of the cows increased when the amount of grain in the ration was increased, which indicates that the feeding of grain stimulates the appetites of cows, at least over short periods of time. The average daily intake of dry matter was 24.39 pounds on roughage alone, 28.16 pounds on limited grain, and 31.78 pounds on the full grain ration. Although there was a significant increase in the total dry matter consumption, when grain was added to the ration, there was a decrease in consumption of dry matter from the roughage portion.

In every period the cattle on roughage alone failed to consume sufficient digestible nutrients to meet the calculated requirements for maintenance and milk production. The average deficiency in digestible

nutrient consumption was 21.89 per cent, while there was an average per cow per day loss in live weight of 0.43 pound. The cattle on limited grain feeding consumed 10.84 per cent less than enough nutrients to meet the calculated requirements, but oddly enough there was an average increase in live weight of 0.11 pound per cow per day.

The cattle consumed almost exactly the amount of nutrients required during the time they were fed the full grain ration. They also gained in weight in these periods.

The average daily production of fat corrected milk (4 per cent butterfat) while the cows were being fed ration A was 30.3 pounds; while being fed ration B it was 34.3 pounds; and while being fed ration C it was 37.2 pounds. Although these figures give evidence of appreciable differences between the ration effects on milk production, they fail to show the true differences.

If there were no carry-over effect on milk yield of one ration to the next, the simple analysis of variance would suffice to discern the true ration effects on production. In the case of total nutrients, the consumption in one period did not appear to have been influenced by the ration given in the previous period, while with milk yield, a carry-over effect is present. Therefore, the simple, unaltered averages fail to give an unbiased estimate of the ration effects on milk production.

Because carry-over effects are present, the average milk yields under each ration must be adjusted to avoid bias. The design employed in this experiment allowed such adjustments to be made, by using a technique known as the method of least squares.

After estimating the residual effects the mean milk yields were adjusted. This adjustment caused some alteration in the magnitude of the original production means, as shown in Table I.

The adjustments reduced the mean yield of the cows fed roughage alone by 19 pounds of milk and increased the mean yield of the cows fed the full grain ration by about the same amount. The adjusted mean yield of the cows when receiving limited amounts of grain did not change materially from the actual mean yield, seemingly because the beneficial

TABLE I  
MEAN YIELDS OF FAT CORRECTED MILK PER COW PER PERIOD OF SIX WEEKS

RATION	UNADJUSTED YIELDS			YIELDS ADJUSTED FOR CARRY-OVER EFFECTS		
	Mean Pounds	Increase over Roughage		Mean Pounds	Increase over Roughage	
		Pounds	Percent- age		Pounds	Percent- age
Roughage alone .....	1270.8			1251.5		
Limited grain .....	1440.3	+169.5	+13.3	1441.7	+190.2	+15.2
Full grain .....	1580.7	+289.9	+22.8	1578.7	+327.2	+26.1
Standard Error .....				±20.3	±28.7	±2.3

carry-over effect from the full grain feeding was cancelled out by the detrimental carry-over effect from feeding roughage alone. Without adjustments for the carry-over effects, the differences between the rations were underestimated by about 11 per cent.

The statistical analysis shows that the design attained a satisfactory degree of precision. The standard error per cow, total of six weeks, was 77.84 pounds, or only 5.5 per cent of the mean, 1,424 pounds; and the differences between the ration means were highly significant.

In order to arrive at some estimate of the relative physiologic efficiency of the rations, the ratios, fat corrected milk produced/total digestible nutrients consumed above maintenance, were calculated. This value was 5.76 for the cattle on roughage alone, 4.09 for those on limited grain, and 3.20 for those receiving the full grain ration. In this measure of physiologic efficiency, the greater values for the high roughage planes of feeding decreased as the lactation period advanced so that by the end of the eighth month of lactation the differences between the rations as measured by this ratio were very slight.

It must be remembered that the animals on the high roughage rations suffered losses in live weight, while those on the full grain ration consumed sufficient nutrients to meet their requirements and gained in live weight, all of which tends to discount the calculated higher "physiologic efficiency" values of the high roughage rations. Hence it is perhaps unfair to ration C to accuse it of being as low, relatively, as the calculated "physiologic efficiency" value suggests.

The comparisons of economy of production on the three different planes of feeding are shown in Table II.

TABLE II  
RETURNS ABOVE FEED COSTS (AVERAGE FOR SIX-WEEK PERIOD) OF DAIRY COWS ON  
THREE PLANES OF FEEDING\*

TIME OF YEAR	Butterfat Per Lb.	Hay Per Ton	Grain Per Ton	Roughage Alone	Limited Grain	Full Grain
March	\$0.31	\$7.96	\$21.05	\$11.16	\$12.30	\$12.13
July	0.28	6.72	20.64	10.16	11.07	10.81
November	0.32	7.18	19.51	11.82	13.21	13.24

\* Prices used are averages of Iowa market prices for the years 1936-40. For all calculations skimmilk was priced at \$0.25 per cwt., and silage at \$4.00 per ton.

Full grain feeding proved to be slightly more profitable than no grain feeding, and the limited grain ration was the most profitable of the three. However, these differences between the rations from the standpoint of returns above feed costs are quite small regardless of the time of year (March, July, or November).

Using the formula developed by Cannon and Espe, and the prices given in the above table, the value of grain in the dairy ration was calculated. The results showed that grain, when fed in limited quantities, was worth slightly more than when fed in full amounts.

### CONCLUSIONS

The double change-over design used in the latter experiment was successful in permitting a thorough analysis and interpretation of the results. The carry-over effects were adequately discerned from the true ration effects.

The results of these trials show that under midwestern price conditions as during the past five to six years, it will pay farmers to include at least a limited amount of grain in their dairy rations. However, if butterfat prices decrease to \$0.25 per pound, or less, and grain prices approach \$30 per ton, many dairy farmers will doubtless find it wise to decrease the amount of grain fed, or eliminate it entirely.

# FERMENTATIVE UTILIZATION OF CASSAVA<sup>1</sup>

JULIAN R. BANZON

From the Department of Chemistry, Iowa State College

Received May 22, 1941

The cassava, *Manihot utilissima*, is a plant possessing quite unusual characteristics. It has no known pests nor enemies; it grows in moist soils, resists extreme droughts, and propagates easily. It is the cheapest source of starch known, and at present the industries have not made much use of the cassava except for this purpose.

A difficult situation has arisen in the Philippines because petroleum resources are almost absent. The importation of liquid fuels has steadily increased despite the extensive employment of ethyl alcohol, either straight or blended, in power tractors, trucks, and busses. The alcohol is manufactured from cane molasses, but the entire molasses output of the country if fermented would hardly meet the domestic fuel requirement.

The cassava as a potential source of alcohol has been suggested, but there has been no large scale development along this line. Being starchy, cassava requires a special treatment, that of saccharification, before it can be acted upon by yeast. The current methods of saccharification make use of acids or malt, both of which are too costly and are inapplicable to local Philippine conditions.

The production of chemicals like butanol, acetone, and ethanol from cassava is also a step towards the industrialization of the country. For these reasons the fermentative utilization of cassava was made the object of the investigation.

## MATERIALS AND METHODS

The cassava samples were obtained as dried chips from peeled and unpeeled roots; the chips were ground to a powder. The mold-bran employed for saccharification was prepared from a strain of *Aspergillus orzyae* and wheat bran according to the method of Underkofer, Fulmer, and Schoene.<sup>2</sup>

In the investigations on alcoholic fermentation the ethanol yield was used as the measure of efficacy of the various experimental treatments. Fermentations were carried out by means of a strain of *Saccharomyces cerevisiae*. The fermented mashes were distilled, and the ethanol content was determined from the specific gravity of the distillates. The calculation of ethanol yield was based on the theoretical equation:  $C_6H_{12}O_6 \rightarrow 2C_2H_5OH + 2CO_2$ . The glucose equivalent of the starch in the samples was determined by the official A. O. A. C. acid hydrolysis method and by the Shaffer-Hartmann titration procedure. Ethanol cor-

<sup>1</sup>Original thesis submitted July 15, 1940. Doctoral thesis number 578.

<sup>2</sup>Underkofer, Fulmer, and Schoene. Indust. Engr. Chem. 31:734-735 (1939).

rections for the inoculum and for the other added materials were evaluated by means of differential fermentations.

In the butyl-acetonic fermentation the experimental mashes were inoculated with the third transfer from a spore culture of a strain of *Clostridium acetobutylicum*. After incubation for 72 hours at 30° C. the fermented mashes were distilled and total solvents in the distillate were determined from the specific gravity and the Christensen-Fulmer equation:

Total solvents, g./100 ml. = 698 (1.000 — sp. gr. 25/25). Due correction was made for inoculum and other materials added. Yield was expressed as grams total solvents per hundred grams of glucose equivalent of sample.

## EXPERIMENTAL RESULTS

### A. ALCOHOLIC FERMENTATION

The saccharification of cassava by means of sulfuric acid was investigated. Acid concentration and the ratio of sample to acid were varied, and the corresponding effect on the conversion of starch to glucose was determined by the Shaffer-Hartmann titration. The acid hydrolysates were neutralized with ammonium hydroxide and were inoculated with yeast. The highest conversion of starch to glucose was obtained when the acid concentration was 0.4 normal and when the ratio of sample to acid was 1:2.5 or 1:3. Yeast fermentation of the acid hydrolysates gave poor yields of ethanol. The yields improved when mold-bran was added and when the dilution was increased. The effect of adding malt, wheat bran, inactivated mold-bran, inactivated malt, and yeast extract to the acid hydrolysates was also studied. Four strains of yeasts were found satisfactory for fermentation. Variations in the cooking pressure, the cooking time, and the acid concentration did not affect ethanol yields to any large extent.

Due to the stiffness of cassava pastes, the usual mashing operation at 55° to 60° C. was found difficult to perform. Various methods of effecting thinning or liquefaction were studied. Cooking the cassava with 0.1 or 0.05 normal sulfuric acid liquefied the pastes sufficiently; the acid, however, had to be neutralized prior to fermentation. The addition of hot water to cassava containing 2 to 3 per cent by weight of dry mold-bran, such that the temperature of the mixture reached 70° C. (gelatinizing temperature) resulted in a mash which liquefied satisfactorily upon standing for 15 to 30 minutes. With this method, however, the amylase in the thinner was rendered unavailable for further enzymatic action due to subsequent cooking of the mash. Thinning was more conveniently accomplished by means of mold-bran equal to 0.5 per cent of weight of sample, made into a suspension in water. The suspension was added to the cassava paste at 75° C. with stirring. Pastes that had cooled down and been reheated gave variable results. Carrying the thinning operation at 65° C. was thoroughly satisfactory and gave consistent results. The

method failed above 75° C. The bacterial enzyme preparation known as "Rapidase" effected liquefaction with ease at 80° C.

Conditions for the saccharification of cassava mash by means of mold-bran were studied. Mash concentrations up to 20 per cent gave good yields of ethanol; highest yield was obtained at a mash concentration of 16 per cent. For ease of working, thinning of the mash with mold-bran suspension prior to saccharification was advantageous. The proportion of mold-bran necessary for saccharification was 7.5 to 10 per cent of the weight of the cassava. In yeast fermentation the volume of the inoculum was found to be not critical and could be as low as 2 per cent of the total volume, although slightly better results were obtained with 6 per cent. The length of time of fermentation was about 72 hours when incubation was at 30° C. The effect of grinding the cassava to a state of fine subdivision and the effect of stirring the mash during saccharification were also studied; the results were inconclusive. The "malting" or saccharifying operation so necessary for the malt process was given careful attention. A study of the effect on final ethanol yields of the temperature of the saccharifying bath demonstrated that the higher the temperature the lower were the yields. A similar study showed that the longer the sample is kept in the bath the lower were the results. The usual "malting" operation was found unnecessary and the fermentation procedure was simplified by the omission of the saccharifying bath. The mold-bran was simply introduced into the previously thinned mash at 30° C. No temperature control was necessary within the range of 30° to 40° C.

The employment of barley malt as a saccharifying agent in the alcoholic fermentation of cassava was found to be unsatisfactory. The effect on ethanol yields of the concentration of the cassava in the mash, the proportion of barley malt, and the ratio of thinner to saccharifier were investigated. The ethanol yields obtained were always low. Increasing the quantity of malt in the mash improved the yields somewhat, but even when the amount of malt was 20 per cent of the weight of the cassava the ethanol yield did not quite reach 70 per cent. Varying the concentration of cassava in the mash did not affect the ethanol yields. The amylase in malt was thought to differ from that in mold-bran, because mashes which had been thinned with mold-bran and saccharified with malt gave higher yields of ethanol than mashes which had been treated with malt exclusively.

#### B. BUTYL-ACETONIC FERMENTATION

The butyl-acetonic fermentation of cassava alone was found to give very poor yields of total solvents. Replacement experiments demonstrated that as much as 80 per cent of corn, the standard substrate for this type of fermentation, could be replaced by cassava and still obtain as good a yield of solvents as from corn alone. Shrimp powder, corn gluten meal, soybean flour, compressed yeast, peptone, and urea were

investigated as possible nutrients when added to cassava mash. Yields of total solvents comparable to those obtained from corn were attained when shrimp powder, corn gluten meal, and soybean flour were incorporated into the mash. The quantity required was about 5 per cent. Shrimp powder was considered particularly promising because of its ready availability in the Philippines.

# CHEMICAL ENGINEERING APPLICATIONS OF A MODIFIED ARCHIMEDEAN SCREW<sup>1</sup>

HERBERT TEMPLETON BATES

*From the Department of Chemical Engineering, Iowa State College*

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The theory of operation of the modified Archimedean screw<sup>2</sup> was extended by allowing for the space occupied by the flights and by determining the optimum angle of operation for the maximum water horsepower. The modified Archimedean screw was shown to be an equilibrium device, which except for frictional losses would operate on a reversible work cycle.

The modified Archimedean screw has the characteristic of lifting relatively large volumes of liquid short distances reliably and efficiently. This makes possible an aeration device consisting of an Archimedean screw with nested sidewalls attached at the bottom to the outer edge of the screw flights and with large flat spiral fins attached to the upper edges of the sidewalls. When the lower end of this aerator is submerged in a liquid and rotated, liquid is raised inside the Archimedean screw and distributed evenly over the faces of the fins in contact with air. The aerator can be applied to any of the aeration processes: water cooling, humidification, or oxidation of chemical solutions. Since the discharge from the fins ordinarily occurs near the bottom edges, windage loss is reduced to a minimum.

In order to study the effects of changes in speed, size, and inclination of the fins on the rate of water cooling, a flat circular disc rotating about an axis through its center was set up. It was found desirable to rotate the disc slowly in a plane near the horizontal for the best rate of water cooling. The cooling range was easily increased by decreasing the water feed rate. An Archimedean aerator design must be a compromise between the best angle of operation for the fins and the best angle for the Archimedean screw.

The heat and mass transfer coefficients for the water-cooling process were calculated by an approximate method from a large number of experimental runs using both the Archimedean aerator and the rotating disc. At normal water flow rates, these coefficients were substantially independent of water feed rate, but they were dependent upon the angle of operation of the disc. The coefficients were considerably larger when the air was circulated with a fan than they were during natural convection alone. At very low water flow rates the heat and mass transfer coefficients were found to decrease sharply to very small values. The

<sup>1</sup>Original thesis submitted June, 1941. Doctoral thesis number 614.

<sup>2</sup>Rollman, W. F. The principle of the archimedean screw in chemical engineering. Doctoral Thesis No. 462. Library, Iowa State College. (1938.) [Unpublished.]

latter phenomenon is presumably due to the failure of some of the assumptions inherent in the mathematical analysis, since the coefficients should be independent of water feed rate.

The Archimedean screw has the characteristic of delivering the same quantity of liquid for each revolution it makes at any fixed angle of operation. This makes possible the development of metering and proportioning devices. Two of these were built, and tested. The first consisted of a spiral of tubing wound over a light steel framework. The tube was clamped to the frame at the bottom, but the remainder of the helix was free to slide. In operation at a fixed angle and at a fixed speed, this pump delivered liquid at a definite rate which could be closely regulated by changing the tension on the helix, thus altering the pitch.

An automatic proportioner was built which operated at a constant speed but at a variable angle of operation. The cradle which supported the rotating Archimedean screw, its drive motor, and its speed reducer was pivoted at the bottom and suspended near the upper end from a counterbalancing boom. The boom was pivoted at the center, and the link connecting the upper end of the boom to the cradle was arranged to keep the boom and cradle parallel at all times. A counterbalancing weight was adjusted near the lower end of the boom to keep the moving parts balanced at all angles.

To control the angle of operation of the Archimedean screw in the automatic proportioner, water was run over a weir, and a float behind the weir was connected by means of a rope and pulleys to the cradle of the screw. The rope was connected to the cradle so as to lift as nearly vertically as possible. Consequently, the mechanical advantage of the float acting on the cradle increased roughly as the weight of water in the Archimedean screw increased. A specially shaped weir was designed in order that the flow over the weir would match the discharge from the Archimedean screw at all but a very low rate of flow.

The automatic proportioner was tested as a chemical feeder for a cold process lime-soda ash water-softening installation. The performance was satisfactory when the speed of rotation of the Archimedean screw was not too high, and when the agitation of the chemical slurry was not sufficient to develop foam on the surface. The inertia of the counterbalancing system was large enough to prevent the proportioner from following rapid changes in the flow rate.

# THE THEORY AND APPLICATION OF TENSOR ANALYSIS<sup>1</sup>

ROBERT EDWIN BEAM

From the Department of Electrical Engineering, Iowa State College

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In an attempt to establish a correspondence between physical phenomena and mental processes, the principles of geometrical representation of algebraic formulas are used. The methods of tensor analysis provide a means of algebraic formulation; the principles of differential geometry provide a suitable method of reasoning. The first five parts of this thesis are devoted to an exposition of those fundamentals of tensor analysis and differential geometry which seem essential from the viewpoint of applicability to engineering problems; the remaining parts are devoted to applications.

The principal application considered centers about the fact that essentially the same geometrical theory represents either mechanical or electrical circuits provided the analogous concepts are properly chosen. The fundamental postulate

$$\frac{D}{Dt} \left( g_{ij} \frac{dx^j}{dt} \right) = X_i, \quad (1)$$

where  $\frac{D}{Dt}$  is the intrinsic or absolute derivative, is assumed to represent

a combined electro-mechanical system after the analogous quantities are once defined. If the quantities  $g_{ij}$  are interpreted as components of an inertial tensor, if the quantities  $\frac{dx^j}{dt}$  are interpreted as components of a

velocity vector, and if the quantities  $X_i$  are interpreted as components of the resultant of the applied forces, then the fundamental postulate represents Newton's second law of motion in tensor form. This postulate can be expanded after introducing\*

$$g_{ij} \frac{dx^j}{dt} = \frac{\delta}{\delta x_t^i} \left( \frac{1}{2} g_{ij} \frac{dx^i}{dt} \frac{dx^j}{dt} \right) = \frac{\delta T}{\delta x_t^i} \quad (2)$$

to obtain

$$\frac{d}{dt} \left( \frac{\delta T}{\delta x_t^i} \right) - \frac{\delta T}{\delta x^i} = X_i, \quad (3)$$

<sup>1</sup> Original thesis submitted August 21, 1940. Doctoral thesis number 595.

\* Here and in the sequel  $x_t = \frac{dx}{dt}$ . Similarly  $\theta_t = \frac{d\theta}{dt}$ ,  $s_t = \frac{ds}{dt}$ ,  $s_{tt} = \frac{d^2 s}{dt^2}$  etc.

Lagrange's equations of motion. Similarly, by introducing the analogous electric circuit concepts, the fundamental postulate represents the performance of an electrical network in tensor form. The broader interpretation results by considering the quantities in the fundamental postulate to represent either electrical or mechanical concepts in an electro-mechanical system.

The fundamental postulate may be referred to either a true-co-ordinate reference system or an intrinsic reference system consisting of an enneuple of congruences. By allowing the fundamental postulate to represent a typical electro-mechanical system, the rotating electrical machine, the performance can be expressed in terms of either true or intrinsic variables. If the reference system is properly chosen, it is possible to obtain the performance equations in a form which is simpler to solve than the initial form. By making the usual idealizations of negligible hysteresis and eddy-current losses of sinusoidal variations of inductances and of two independent rotor currents, the performance equations of a three-phase induction motor are expressed along a particular intrinsic reference system in a form which involves only constant coefficients. The transformation coefficients

$$\left[ \frac{\partial q^i}{\partial s^j} \right] = \begin{array}{cccccc|c} s^1 & s^2 & s^3 & s^4 & s^5 & \theta & \\ \hline 1 & 0 & 1 & 0 & 0 & 0 & q^1 \\ -\frac{1}{2} & +\frac{\sqrt{3}}{2} & 1 & 0 & 0 & 0 & q^2 \\ -\frac{1}{2} & -\frac{\sqrt{3}}{2} & 1 & 0 & 0 & 0 & q^3 \\ 0 & 0 & 0 \cos \theta & \sin \theta & 0 & 0 & q^4 \\ 0 & 0 & 0 \cos(\theta+120) & \sin(\theta+120) & 0 & 0 & q^5 \\ 0 & 0 & 0 \cos(\theta-120) & \sin(\theta-120) & 0 & 0 & q^6 \\ 0 & 0 & 0 & 0 & 0 & 1 & \theta \end{array} \quad (4)$$

where the  $s^i$  are intrinsic variables and the  $q^i$  are true-co-ordinate variables, are used to transform the initial tensor components  $g_{ij}$ ,  $R_{ij}$ , and  $e_j$ . The inductance and inertial tensor components

(5)

$$\begin{matrix}
 & q^1 & q^2 & q^3 & q^4 & q^5 & q^6 & q^7 \\
 & L_a & -M_a & -M_a & M \cos \theta & M \cos (\theta + 120) & M \cos (\theta - 120) & q^1 \\
 & -M_a & L_a & -M_a & M \cos (\theta - 120) & M \cos \theta & M \cos (\theta + 120) & q^2 \\
 & -M_a & -M_a & L_a & M \cos (\theta + 120) & M \cos (\theta - 120) & M \cos \theta & q^3 \\
 & M \cos \theta & M \cos (\theta - 120) & M \cos (\theta + 120) & L_b & -M_b & -M_b & q^4 \\
 & M \cos (\theta + 120) & M \cos (\theta - 120) & -M_b & L_b & -M_b & 0 & q^5 \\
 & M \cos (\theta - 120) & M \cos (\theta + 120) & M \cos \theta & -M_b & -M_b & L_b & q^6 \\
 & 0 & 0 & 0 & 0 & 0 & 0 & q^7
 \end{matrix}$$

$\mathbf{g}_{ij} =$

transform to

$$\begin{array}{ccccccccc}
 & s^1 & s^2 & s^3 & s^4 & s^5 & s^6 & & \\
 & \downarrow & \downarrow & \downarrow & \downarrow & \downarrow & \downarrow & & \\
 \left[ \begin{array}{cccccc}
 \frac{3}{2}(L_a+M_a) & -\frac{\sqrt{3}}{2}M_a & -6M_a & \frac{9}{2}M & 0 & 0 & 0 & s^1 \\
 -\frac{\sqrt{3}}{2}M_a & \frac{3}{2}(L_a+M_a) & -\sqrt{3}M_a & 0 & \frac{9}{2}M & 0 & 0 & s^2 \\
 -6M_a & -\sqrt{3}M_a & 3(L_a+2M_a) & 0 & 0 & 0 & 0 & s^3 \\
 \frac{9}{2}M & 0 & 0 & \frac{3}{2}(L_b+M_b) & -\frac{\sqrt{3}}{2}M_b & 0 & 0 & s^4 \\
 0 & \frac{9}{2}M & 0 & -\frac{\sqrt{3}}{2}M_b & \frac{3}{2}(L_b+M_b) & 0 & 0 & s^5 \\
 0 & 0 & 0 & 0 & 0 & 0 & j_r & \theta
 \end{array} \right] \quad (6)$$

$\rightarrow$

$s'_{ij} =$

In (5) and (6) the variables  $q^1, q^2$ , and  $q^3$  are stator variables corresponding to phases 1, 2, and 3, respectively; and  $q^4, q^5$ , and  $q^6$  are the rotor variables corresponding to phases 4, 5, and 6, respectively; also  $q^7$  is equal to  $\theta$ , the angular position of the ratio. Similarly, the resistance tensor components

$$R_{ij} = \begin{array}{|c|c|c|c|c|c|c|c|} \hline & q^1 & q^2 & q^3 & q^4 & q^5 & q^6 & q^7 \\ \hline i & R_a & 0 & 0 & 0 & 0 & 0 & 0 \\ \downarrow & 0 & R_a & 0 & 0 & 0 & 0 & 0 \\ & 0 & 0 & R_a & 0 & 0 & 0 & 0 \\ R_{ij} = & 0 & 0 & 0 & R_b & 0 & 0 & 0 \\ & 0 & 0 & 0 & 0 & R_b & 0 & 0 \\ & 0 & 0 & 0 & 0 & 0 & R_b & 0 \\ & 0 & 0 & 0 & 0 & 0 & 0 & R_r \\ \hline & q^1 & q^2 & q^3 & q^4 & q^5 & q^6 & q^7 \\ \hline \end{array} \quad (7)$$

j →

transform to

$$R'_{ij} = \begin{array}{|c|c|c|c|c|c|c|c|} \hline & s^1 & s^2 & s^3 & s^4 & s^5 & \theta \\ \hline i & \frac{3}{2}R_a & 0 & 0 & 0 & 0 & 0 \\ \downarrow & 0 & \frac{3}{2}R_a & 0 & 0 & 0 & 0 \\ & 0 & 0 & 3R_a & 0 & 0 & 0 \\ R'_{ij} = & 0 & 0 & 0 & \frac{3}{2}R_b & 0 & 0 \\ & 0 & 0 & 0 & 0 & \frac{3}{2}R_b & 0 \\ & 0 & 0 & 0 & 0 & 0 & R_r \\ \hline & s^1 & s^2 & s^3 & s^4 & s^5 & \theta \\ \hline \end{array} \quad (8)$$

j →

Further, the three applied voltages  $e_1, e_2, e_3$ , and the applied torque  $e_7$  transform to

$$e'_1 = e_1 - \frac{1}{2}(e_2 + e_3),$$

$$e'_2 = \frac{3}{2}(e_2 - e_3), \quad (9)$$

and

$$e'_7 = e_7.$$

The transformed quantities  $g'_{1j}$ ,  $R'_{1j}$ , and  $e'_i$  are substituted in the following dynamical equation, expressed in terms of intrinsic reference variables  $s^i$ , to obtain the equations of performance of the induction motor:

$$\begin{aligned} R'_{ak}s_t^a + g'_{ak}s_{tt}^a + & \left[ \left( \frac{\partial g'_{ak}}{\partial s^\nu} - \frac{1}{2} \frac{\partial g'_{a\gamma}}{\partial s^k} \right) \right. \\ & \left. + g'_{a\beta} \left( \frac{\partial}{\partial q^m} \frac{\partial s^\beta}{\partial q^i} - \frac{\partial}{\partial q^i} \frac{\partial s^\beta}{\partial q^m} \right) \frac{\partial q^i}{\partial s^k} \frac{\partial q^m}{\partial s^\nu} \right] \\ s_t^a s_t^\gamma &= e'_k. \end{aligned} \quad (10)$$

For each value of  $k$  in equation (10) there is a sum on  $\alpha$ ,  $\beta$ ,  $\gamma$ ,  $i$ , and  $m$ . The result of the substitution is the following set of performance equations:

$$\begin{aligned} \frac{3}{2} \left[ R_a + (L_a + M_a)p \right] s_t^1 - \frac{\sqrt{3}}{2} M_a p s_t^2 - 6 M_a p s_t^3 + \\ \frac{9}{2} M p s_t^4 = e_1 - \frac{1}{2}(e_2 + e_3) \end{aligned} \quad (11)$$

$$\begin{aligned} - \frac{\sqrt{3}}{2} M_a p s_t^1 + \frac{3}{2} \left[ R_a + (L_a + M_a)p \right] s_t^2 - \sqrt{3} M_a p s_t^3 + \\ \frac{9}{2} M p s_t^5 = \frac{3}{2}(e_2 - e_3) \end{aligned} \quad (12)$$

$$\begin{aligned} - 6 M_a p s_t^1 - \sqrt{3} M_a p s_t^2 + 3 \left[ R_a + (L_a - 2M_a)p \right] s_t^3 = \\ e_1 + e_2 + e_3 \end{aligned} \quad (13)$$

$$\begin{aligned} \frac{9}{2} M p s_t^1 + \frac{3}{2} \left[ R_b + (L_b + M_b)p \right] s_t^4 + \frac{\sqrt{3}}{2} M_b p s_t^5 \\ + \frac{3}{2}(L_b + M_b) \theta_t s_t^5 + \frac{9}{2} M \theta_t s_t^2 = 0 \end{aligned} \quad (14)$$

$$\frac{9}{2} M p s_t^2 - \frac{\sqrt{3}}{2} M_b p s_t^4 + \frac{3}{2} \left[ R_b + (L_b + M_b)p \right] s_t^5$$

$$-\frac{3}{2}(L_b + M_b) \theta_t s_t^4 - \frac{9}{2} M \theta_t s_t^1 = 0 \quad (15)$$

$$(R_r + j_r p) \theta_t = e_7 \quad (16)$$

These equations represent the steady-state and transient performance of a three-phase induction motor; they are somewhat similar to those obtained by Stanley<sup>2</sup> who used a non-tensor type of transformation. These equations may be given a non-Riemannian geometrical interpretation.

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<sup>2</sup>STANLEY, H. C. An analysis of the induction motor. Trans. A.I.E.E. Vol. 57, supplement. 1938.

# IONIC EXCHANGE MATERIALS AND THEIR USES<sup>1</sup>

RICHARD ERNEST BENSON

*From the Department of Chemical Engineering, Iowa State College*

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Commercial gel-type zeolites are produced by dehydrating an alkaline aluminosilicate gel by one of the following three methods: (1) drying; (2) filter pressing and drying; and (3) freezing, thawing, and then drying. After the gel has been dehydrated to the desired moisture content, it is washed, screened, the oversized particles crushed and rescreened, and all the fines discarded. In order to eliminate several of these operations and to decrease the magnitude of the others, a method of dehydration by means of hydraulic pressure has been developed. The process is believed to give a product with desirable properties, and to effect economies in production.

Properties of zeolites, such as chemical composition, exchange capacity, particle size, resistance to abrasion, and resistance to decrepitation, are more or less closely related to one another and to the particular method of manufacture. Because the greater percentage of the siliceous base-exchange substances currently marketed have a  $\text{SiO}_2 : \text{Al}_2\text{O}_3$  ratio of 5 : 1, this ratio was employed in order to have a product whose chemical composition would be readily accepted.

The zeolite gels were prepared by mixing a dilute solution of sodium aluminate with an equal volume of a dilute solution of sodium silicate. An  $\text{Al}_2\text{O}_3$  concentration of 0.12 gram mol per liter was adopted because at higher concentrations the resulting solution gelled so rapidly that good mixing—essential for high exchange capacity—was impossible. This maximum concentration of reactants was employed not only because it increased the yield of product per unit of volume of gel handled, but also because its use produced the gel which had the best structure and the one which could be most easily dehydrated by hydraulic pressure. The gel was formed in a thin sheet on a filter cloth. Layers of gel separated by filter cloth were stacked one above the other. An extra cloth was placed over the top layer of gel. By carefully controlling the rate at which the pressure was increased, practically no gel was lost at the open sides when the dimensions of the filter cloths were such that they provided a 1-inch border around the respective layers of gel which they separated. The exchange capacity of pressed, but undried, zeolites increased with the pressure up to 6,500 pounds per square inch. The capacity of pressed zeolite dried to a 50 per cent moisture content was not influenced by the hydraulic pressure employed, and was equal in capacity to pressed but undried zeolites subjected to a pressure greater than 6,500 pounds per

<sup>1</sup> Original thesis submitted July 13, 1940. Doctoral thesis number 575.

square inch. The durability and strength of all pressed zeolites were increased to such an extent that it was desirable to dry the pressure-dehydrated zeolite to a 50 per cent moisture content. The amount of water removed by drying was less than 10 per cent of the total moisture removed from the original gel.

The filtrate, which constituted more than 90 per cent of the moisture removed from the gel, contained unreacted sodium aluminate and sodium silicate. However, it was not feasible to re-use this mother liquor in preparing the dilute solutions of sodium aluminate and sodium silicate from the commercial materials, because its use caused a decrease in the  $\text{SiO}_2:\text{Al}_2\text{O}_3$  ratio in the product, a decrease in the gel structure, a decrease in the strength of the resulting zeolite, and an increase in the concentration of the reactants in the filtrate. The concentration of the reactants in the filtrate depended upon the length of time that the gel was aged before it was subjected to dehydration by hydraulic pressure. In the following table are recorded the concentrations of the reactants in the filtrate when a gel containing 0.12 gram mols of  $\text{Al}_2\text{O}_3$  and 0.60 gram mols of  $\text{SiO}_2$  per liter was dehydrated after it had been aged various lengths of time.

Time (minutes)	5	8	20	40	43
$\text{SiO}_2$ (g. Mol/l) .....	0.013	0.0076	0.011	0.032	0.037
$\text{Al}_2\text{O}_3$ (g. Mol/l) .....	0.013	0.0103	0.006	0.0014	0.001

With the delivery price of sodium aluminate at \$0.065 per pound, and that of sodium silicate at \$0.009 per pound, the optimum time of aging was found to be 31 minutes. The zeolite produced from a gel of the above composition after the gel has been aged 31 minutes had a  $\text{SiO}_2:\text{Al}_2\text{O}_3$  ratio of 4.9 : 1. In order to operate with the minimum loss in the filtrate and still produce a zeolite with a  $\text{SiO}_2:\text{Al}_2\text{O}_3$  ratio slightly greater than 5 : 1, the ratio of  $\text{SiO}_2:\text{Al}_2\text{O}_3$  in the gel was increased from 5 : 1 to 5.2 : 1.

It was observed that the particle size of the pressed zeolite could be closely controlled by the solid content per unit area of the sheet of pressed gel. With the composition of the gel fixed, the solids per unit area varied directly with the thickness of the gel. Although the pressed mineral, after drying, could be removed from the filter cloth by means of a brush, this method was tedious and had a tendency to increase the percentage of "fines," material passing a fifty mesh screen. By incorporating a metallic screen into each layer of gel, the pressed zeolite could be easily removed from the cloths. By drying the pressed zeolite to a 50 per cent moisture content, the zeolite could be removed simply by vibrating the screen. A mineral of exceptionally uniform size was obtained entirely free of over-sized particles and containing less than 3 per cent "fines." By varying the thickness of the gel, a marketable mineral of any desired average particle size could be obtained directly with-

out resorting to any method of removing the over-sized or under-sized particles or crushing the over-sized particles.

Because the pressed sheets of gel were approximately 1 millimeter in thickness, the partially dehydrated gel could be dried quite rapidly under ordinary drying conditions. It was found that the conditions of drying, as well as the extent of drying, very markedly influenced the exchange capacity of the resulting zeolite, and that only by carefully controlling the conditions under which the pressed gels were dried could minerals of high exchange capacity be obtained. It was observed that the rate of drying during the initial stages could be quite rapid but that the latter stages of drying had to be performed more slowly. Zeolites obtained from pressed gels which were properly dried had exchange capacities of 11,000 grains per cubic foot as compared to 9,000 grains per cubic foot of two of the better known commercial zeolites of the same chemical composition and grain size, and as compared to 1,000 to 4,000 grains per cubic foot of zeolites obtained from pressed zeolites which were dried too rapidly. It is thought that the difference in exchange capacity between commercial zeolites and pressed zeolites was caused only partially by the more careful processing which was possible because of the batch sizes employed. A portion of the difference in exchange capacity was caused presumably by first dehydrating the gel by hydraulic pressure before drying.

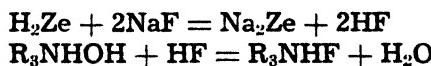
Even though it is known that ionic exchange is a surface phenomenon, and, as a result, the exchange capacity is almost directly proportional to the surface exposed, the above assumption appeared more valid when it was observed that pressed zeolites were approximately 8.2 per cent more dense than commercial zeolites. This greater density is a disadvantage in that the chemical costs would be greater for pressed zeolites than for present commercial zeolites. Also, the freight costs per cubic foot of zeolite would be greater. These disadvantages are compensated by the increase in the amount of alumino-silicate per unit volume which is available to resist the dissolving action of the water, as well as by any increase in exchange capacity which can be attributed to the increased density of the pressed zeolites.

After the pressed zeolites had been immersed in water, they were found to be extremely resistant to decrepitation. Because of the large number of fissures that occurred in the sheet of pressed gel during drying, and because handling during the latter stages of processing was insufficient to completely sever the existing fixtures, an apparently large degree of decrepitation occurred the first time the pressed zeolites were wetted. However, repeated drying and wetting of the pressed zeolite produced only limited additional decrepitation. It was observed that the apparently large initial decrepitation could be almost entirely eliminated by drying the pressed gel in two stages. In the first stage, the pressed gel was dried sufficiently to permit the removal of the mineral from the screens. In the second stage, the partially dried zeolite, each particle of

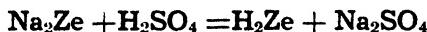
which was then free to contract in all directions, was dried to the desired moisture content of 50 per cent. Two-stage drying decreased the apparent decrepitation, and as a result increased the average particle size of the product.

The hydraulic pressure method of manufacture had one other advantage, namely: washing the excess alkalinity from the product was simplified in that a large portion of the excess caustic already had been removed in the filtrate. In addition, the pressed gel was obtained in a thin but rather rigid sheet which could be readily and economically washed before it was dried.

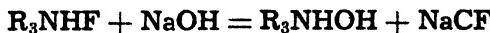
During the study on the properties and characteristics of pressed zeolites, no new uses were developed for siliceous zeolites. However, three new uses which were applicable to carbonaceous exchange substances were developed. The most promising use was the removal of fluorine from natural fluoride-bearing water by dual ionic exchange. The water was filtered through a bed of Zeo-Karb, a commercial carbonaceous zeolite furnished by the courtesy of the Permutit Co. The Zeo-Karb was operated in the hydrogen cycle so that during the filtering operation nearly all of the cations were replaced by hydrogen ions with the formation of the corresponding acids. This acid effluent was then filtered through a bed of Nalcite B, a commercial anionic exchange substance furnished by courtesy of the National Aluminate Corp. The Nalcite B was operated in the basic cycle so that during the filtering operation the anions were replaced by hydroxyl ions with the formation of water. The reactions involved may be represented as follows:



When the carbonaceous zeolite was exhausted it was regenerated by means of a 2 to 5 per cent acid solution.



The anionic exchange material was regenerated by means of a 0.5 per cent caustic solution.



By the above dual ionic exchange process, the fluorine content of a natural fluoride-bearing water was reduced from 9 to less than 2 p.p.m. By subjecting this effluent containing less than 2 p.p.m. of fluorine to the same treatment, the fluorine concentration was reduced to 0.25 p.p.m.

The extraction of casein from milk by means of a hydrogen zeolite was partially developed. Fat-free milk was filtered through Zeo-Karb, operated in the hydrogen cycle in order to remove the various ash-forming cations and substitute hydrogen ions in their place. Even when the milk was diluted with 20 volumes of water and cooled to 2° C., most of the casein precipitated upon the particles of Zeo-Karb. The

Zeo-Karb was regenerated by treating a known volume of the material with 20 volumes of 5 per cent hydrochloric acid. The regenerated material was then washed with distilled water until the effluent was free from chloride ions before it was used to extract casein from milk.

Dilute caustic solutions were prepared from sodium chloride and lime by means of Nalcite A, a carbonaceous base-exchange substance furnished by courtesy of the National Aluminate Corp. A saturated lime solution was filtered through a bed of Nalcite B which had previously been regenerated with a solution of sodium chloride. Because of the effect of temperature upon the solubility of lime, the normality of the effluent varied between 0.027 and 0.03. The neutralized effluent gave a zero test for hardness when treated with a standard soap solution.

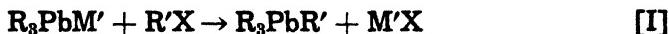
# UNSYMMETRICAL ORGANOLEAD COMPOUNDS<sup>1</sup>

ERNEST BINDSCHADLER

From the Department of Chemistry, Iowa State College

Received June 10, 1941

This research was undertaken to develop methods for the preparation of unsymmetrical organolead compounds of increased water solubility. It is believed that such compounds may be of value in cancer therapy. A method investigated was the reaction of organolead-metal compounds with organic halides<sup>2</sup>.



Investigations were also carried out to develop an improved procedure for the preparation of the organolead-metal compounds.



A third part of the problem was concerned with the arrangement of organic groups in a series according to their ease of cleavage from unsymmetrical organolead compounds.

This investigation showed that the cleavage of groups from an organolead compound [equation II] was a general reaction and was not restricted to certain groups. The following groups were cleaved from appropriate organolead compounds: methyl, ethyl, *n*-butyl, *s*-butyl, allyl, benzyl, phenyl, and *p*-dimethylaminophenyl.

The direct cleavage of organolead compounds by sodium was developed to the stage where it is now the best method available for the preparation of both triphenyllead-sodium and triethyllead-sodium. The formation of sodamide in this method is somewhat of a disadvantage. However, this disadvantage is not serious, because the solution of organolead-sodium compound can be filtered to remove the insoluble sodamide. The sodamide can also be destroyed by the addition of ammonium bromide, and the triphenyllead-sodium is not affected as long as an excess of ammonium bromide is avoided. However, when ammonium bromide was added to a mixture of triethyllead-sodium and sodamide, an appreciable amount of the triethyllead-sodium was destroyed, even though an excess of ammonium bromide was not used. Often it is not necessary to destroy the sodamide. For example, if the solution of organolead-sodium compound is to be used to prepare an unsymmetrical organolead compound [equation I], interference by sodamide may be avoided by the addition of at least two equivalents of the organic halide.

<sup>1</sup>Original thesis submitted June, 1941. Doctoral thesis number 624.

<sup>2</sup>Gilman and Bailie, J. Am. Chem. Soc., 61, 731 (1939).

The preparation of the first unsymmetrical organolead-sodium compounds is reported in this paper. These compounds were formed by the cleavage of unsymmetrical organolead compounds by sodium. For example, diethylphenyllead-sodium was prepared by the cleavage of triethylphenyllead by sodium in liquid ammonia. These unsymmetrical organolead-sodium compounds reacted normally with alkyl halides. However, if one of the groups was either an allyl or benzyl group, the organolead-sodium compound appeared to be unstable even at  $-70^{\circ}$ .

The cleavage of organolead compounds by sodium in liquid ammonia was quite rapid, but the speed of the reaction was markedly influenced by changing the solvent. Tetraethyllead was cleaved immediately in a solvent consisting of a mixture of ether and liquid ammonia, but it was not cleaved before 14 minutes in liquid ammonia alone. On the other hand, tetraphenyllead was cleaved at about the same rate in liquid ammonia as in ether-liquid ammonia. Higher yields of both triphenyllead-sodium and triethyllead-sodium were obtained in ether-liquid ammonia than in liquid ammonia alone. Higher yields were also obtained in the more dilute solutions.

The rate of cleavage of tetraphenyllead by sodium was decreased to a pronounced extent when the reaction mixture was cooled to  $-70^{\circ}$ . The rate of cleavage of tetraethyllead was not decreased appreciably at  $-70^{\circ}$ , however.

The order of decreasing ability of the metals to cleave tetraphenyllead is K, Na > Li, Ca, Sr > Ba. However, calcium, lithium, and sodium cleaved tetraethyllead with approximately equal effectiveness.

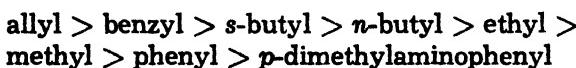
Several attempts to prepare diphenyllead-disodium by the direct cleavage of two phenyl groups from tetraphenyllead were unsuccessful. If the diphenyllead-disodium did form, it was produced in small amounts and was unstable under the conditions of the experiment. However, diethyllead-disodium was prepared by the cleavage of two ethyl groups from tetraethyllead. An ether-liquid ammonia solution of sodium was used as the cleaving agent.

Repeated attempts to prepare triphenyllead-metal compounds free of ammonia were unsuccessful. It was found that tetraethyllead was not cleaved by sodium in ether, and triphenylbenzyllead was not cleaved by sodium amalgam in ether. The binary mixture ( $Mg + MgI_2$ ) did not cleave triphenylbenzyllead, but it did cause this lead compound to undergo a redistribution reaction. In these studies, it was observed that the presence of liquid ammonia was not essential for the production of triethyllead-sodium from tetraethyllead. When the liquid ammonia was replaced by a stream of ammonia gas, and ether was used as a solvent, the cleavage proceeded as usual but was more difficult to control than if the reaction was run in liquid ammonia-ether solvent. The cleavage did not take place, however, when either dimethylaniline or triethylamine was used in place of ammonia.

The effect of various factors on the efficiency of the coupling reaction [equation I] were tested. Steric hindrance caused by the organic groups either in the organolead-sodium compound or in the organic halide seemed to decrease the efficiency of the coupling reaction. Since triethyllead-sodium was more reactive than triphenylead-sodium, more complete coupling was usually obtained when the organic halide was treated with triethyllead-sodium than when it was treated with triphenylead-sodium. In a study of the influence of a branched chain in the organic halide, it was found that triethyllead-sodium coupled completely with *n*-butyl bromide, more slowly and less completely with *s*-butyl bromide, and not at all with *t*-butyl bromide.

The coupling reaction was tried with only one dihalide, ethylene dibromide. This compound did not couple with triphenylead-sodium, but a good yield of hexaphenyldilead was produced. This method can be recommended as a convenient and rapid means for the preparation of hexaphenyldilead, although the yield was lower than that obtained by treatment of triphenylead chloride with sodium in liquid ammonia. The extent of coupling was also influenced markedly by the nature of the organic group in the organic halide. In general, triphenylead-sodium did not couple appreciably with aryl halides. However, it coupled well with the aliphatic halides if a large excess of the halide were present, and it coupled satisfactorily with the more reactive organic halides like benzyl chloride if only a small excess of the halide were present. Triethyllead-sodium coupled well with both alkyl and aryl halides unless the halides were capable of undergoing dehydrohalogenation readily.

Investigations were made on the competitive cleavage of various organic groups from unsymmetrical organolead compounds. The following series, based on the decreasing ease of cleavage of groups from organolead compounds by sodium, was obtained.



This series is almost the complete reverse of that obtained from numerous similar studies formerly made on the cleavage of unsymmetrical organomercury compounds by hydrogen chloride and on the cleavage of unsymmetrical organolead compounds by hydrogen halides, halogens, nitric acid, silver nitrate, thallium chloride, and mercuric chloride.

Series of groups based on the ease of cleavage of organometallic compounds are of value in the prediction of relative reactivities. A reliable series is obtained only when each of the groups is cleaved by the same mechanism. It is believed that hydrogen halides cleave the allyl group by a mechanism different from that involved in the cleavage of other groups from organometallic compounds. Likewise it is believed that bromine cleaves the benzyl group by a mechanism different from that involved in the cleavage of other groups by bromine. However,

sodium appears to cleave all of the groups by the same mechanism; therefore, the sodium cleavage series is thought to be a more reliable comparison between the organic groups than either the bromine cleavage series or the hydrogen chloride cleavage series.

For the identification of some of the products it was necessary to prepare three new organolead compounds. The physical properties of these new organolead compounds are: for diethyl-*n*-butylmethyllead, b.p. 67° (5 mm.),  $n_D^{20}$  1.5125, and  $d_4^{20}$  1.5817; for diethylmethylphenyllead, b.p. 132° (15 mm.) and  $d_4^{20}$  1.7025; and for diethyl-*s*-butylmethyllead, b.p. 87° (10 mm.),  $n_D^{20}$  1.5180, and  $d_4^{20}$  1.5892.

# FRACTIONATION OF STARCH<sup>1</sup>

CARLYLE G. CALDWELL

From the Department of Chemistry, Iowa State College

Received May 22, 1941

## INTRODUCTION

There is considerable evidence in the literature to the effect that starch does not consist simply of threadlike molecules of glucopyranose units joined by alpha-1,4 glucosidic links.

The viscosity studies of Staudinger and Eilers (1) have indicated that starch, in contrast to cellulose, does not consist of long, threadlike molecules but possesses a relatively large molecular weight with a short chain length. Hirst and Young (2) have disaggregated viscous methyl starches by heating with oxalic acid in glycerol. The disaggregated derivatives showed the same proportion of non-reducing end groups (2, 3, 4, 6-tetramethyl glucose) as did the original starch, but the amount of dimethyl glucose which was equal to that of the tetramethyl glucose for the original viscous derivatives was now very small. These authors concluded that starch is built up of 30 glucose unit chains arranged in a branched-chain structure. Such a structure would require an amount of dimethyl glucose equivalent to that of the tetramethyl glucose to be isolated from the hydrolysis products of methyl starch.

Starch has been fractionated into amylose and amylopectin by physical and chemical means. These fractions have different properties, but their proportion, even in one kind of starch, is uncertain. Beta-amylase converts starch into 60 per cent of the theoretical amount of maltose and leaves 25 to 30 per cent of limit or residual dextrans, resistant to the further action of the enzyme (3). The enzyme of *Bacillus macerans* digests starch to result in 15 to 25 per cent of crystalline dextrans which have been formulated as large rings containing five and six glucose units joined by alpha-1,4 linkages (4).

## EXPERIMENTAL

This work reports a study of three methods of fractionating starch: electrodialysis, freezing, and enzyme digestion. In connection with electrodialysis, the phenomenon of retrogradation was investigated. A comparative study of the limit dextrans arising from ordinary cornstarch and from waxy cornstarch was made in an attempt to throw some light on the question of the heterogeneity of starch.

This study has been primarily concerned with cornstarch. The red-iodine-coloring starch from waxy corn was used for comparative purposes throughout in the hope that some difference between the two would give a clue to the question of the heterogeneity of starch.

<sup>1</sup>Original thesis submitted August 19, 1940. Doctoral thesis number 594.

*Fractionation of Starch by Electrodialysis.* A method of determining the degree of fractionation of starch pastes by electrodialysis has been used which depends on the determination of the concentration of starch in the supernatant liquid after a single dialysis. The amount of soluble fraction is given by the product of this concentration and the total volume of paste used. With cornstarch paste heated at 100° C. and homogenized, fractionation resulted in 40 per cent of amylose and 60 per cent of amylopectin.

Increased temperature of preparing cornstarch pastes for electrodialysis resulted in increased amounts of the soluble fraction or amylose only insofar as it brought about greater disorganization of the starch granules. In the temperature range of 80° to 130° C. the soluble fraction increased from 14 per cent to 30 per cent. However, for cornstarch pastes in which the granules had been ruptured by homogenization the soluble fraction decreased from 40 per cent to 30 per cent over the temperature range of 100° to 130° C.

The reducing values of the soluble and insoluble fractions of cornstarch obtained by electrodialysis indicated that the insoluble fraction has a relatively greater average mole size than that of the soluble fraction. It was pointed out that reducing value has no significance as to molecular weight except for comparative purposes. The solutions of the so-called soluble fraction were quite cloudy indicating that this fraction is not truly soluble. In line with this observation, the conclusion was drawn that the degree of fractionation of cornstarch pastes by electrodialysis depends for the most part on the presence of polar groups, i. e., phosphoric and fatty acids. The effect of the temperature of preparing the pastes on the degree of fractionation was attributed to a change in the colloidal state of the starch at the higher temperatures. Waxy cornstarch cannot be separated by electrodialysis showing that it does not contain the insoluble fraction found in ordinary cornstarch by electrodialysis. Dry-ground cornstarch gave considerably more of the soluble fraction by electrodialysis (80 per cent) than did the original starch. This was due to the hydrolytic breakdown during grinding, as was shown by the increased reducing power of the ground starch.

*Fractionation of Starch by Freezing.* The degree of fractionation of starch pastes by freezing was shown to vary with the kind of starch and the treatment of the starch before freezing. Cornstarch pastes gave about 5 per cent soluble fraction. Its solutions were water-clear, and it had a relatively smaller average mole size than the original starch on the basis of reducing value. The amylose of cornstarch obtained by electrodialysis was recovered in 95 per cent yields by freezing, upholding the conclusion that it is not truly soluble. Waxy cornstarch pastes could not be fractionated by freezing. A paste of dry-ground cornstarch, which had suffered considerable degradation as indicated by its reducing value, gave more of the soluble fraction (20 per cent) by freezing than did the original starch. The conclusion was drawn that fractionation of starch pastes by

freezing is more or less dependent on the distribution of mole sizes in the pastes, and the fractions have no particular relation with the fractions obtained by electrodialysis.

*Retrogradation of Starch Pastes.* Retrogradation of cornstarch paste was concluded to have little, if any, effect on its fractionation by electrodialysis. Under the conditions of time and temperature involved with electrodialysis, only slight aging would occur as measured by increased resistance to saliva digestion. The results of the aging experiments were in harmony with the view which pictures retrogradation as a gradual desolvation and consequent precipitation of the large, difficultly soluble molecules which are solvated at the temperatures of preparing the pastes. Pastes of waxy cornstarch and of dry-ground cornstarch did not retrograde, suggesting that their average mole sizes are small enough so that the process of desolvation does not produce appreciable quantities of insoluble material on standing at low temperatures.

*Fractionation of Starch by Digestion with Beta-Amylase.* Fractionation of ordinary cornstarch and of waxy cornstarch by beta-amylase digestion to give rise to the residual or limit dextrans was carried out. The yields of the redigested dextrans amounted to from 30 to 40 per cent of the original starches; the waxy cornstarch dextrin was obtained in slightly greater yields (about 10 per cent) than was the ordinary cornstarch dextrin. These dextrans characteristically showed resistance to further digestion by beta-amylase, low reducing power and retention of the ability to color with iodine. The ordinary cornstarch dextrin was heterogeneous as shown by its fractionation, by alcohol precipitation, by freezing, and by electrodialysis. It consisted of a soluble, red-iodine-coloring fraction and of a difficultly soluble, blue-iodine-coloring fraction. The waxy cornstarch dextrin consisted entirely of a soluble, red-iodine-coloring material.

*Characterization of the Limit Dextrans.* Characterization of representative samples of the cornstarch dextrin and of the waxy cornstarch dextrin by measurements of reducing value, optical rotation, molecular weight of the acetate by the Rast camphor method and by Staudinger's viscosity method and measurement of oxidation with periodic acid revealed no marked difference between these dextrans. However, on the basis of the differences in solubility and iodine colors, the view was adopted that the resistance to digestion by beta-amylase is due to some structural feature of these dextrans rather than to size or solubility. The groups responsible for impedance to beta-amylase action were considered to be concentrated in the limit dextrans.

An estimated chain length of 67 glucose units was found for cornstarch by hydrolysis of the methylated starch and estimation of the proportion of 2, 3, 4, 6-tetramethyl glucose resulting. This value is subject to the reservation that considerable error in the determination of such small amounts of tetramethyl glucose is probable. Hydrolysis of the completely methylated limit dextrin obtained from cornstarch resulted in an

amount of tetramethyl glucose corresponding to an estimated chain length of 9.5 glucose units. This must be erroneous if the dextrin does not consist of straight-chain molecules as is suggested by its resistance to further digestion by beta-amylase. On the same grounds, estimation of the reducing end groups and determination of molecular weight by viscosity measurements would give false values.

Failure to find evidence for any increase in the amount of dimethyl glucose from the methylated cornstarch limit dextrin over that from the methyl cornstarch leads to the conclusion that structures which might give rise to dimethyl glucose, i. e., branched chains, etc., are either absent or are ruptured in the process of methylation.

Evidence that the limit dextrans do not consist of straight-chain molecules and that either one or both of the hydroxyl groups on carbons two and three of the glucose units are involved, was found from a study of the periodic acid oxidation of the limit dextrans. The rate of oxidation was shown to slow up when about 60 per cent of the theoretical amount of periodic acid was consumed. The theoretical value is based on the presumption of a glycol grouping on carbons two and three of all the glucose units. The dextrans oxidized with the theoretical quantity of periodic acid and recovered in nearly 100 per cent yields took up only 60 to 70 per cent of the theoretical amount of hydrogen on catalytic reduction. This supports the conclusion that from 30 to 40 per cent of the glucose units in the limit dextrans do not have a free hydroxyl group on both carbons two and three. The fact that the oxidized dextrans can be precipitated with iodine suggests that it is the structures associated with these blocked glucoses which are responsible for the iodine coloration of the limit dextrans and of starch. The interesting possibility is suggested for further investigation that the crystalline Schardinger dextrans can be obtained from the limit dextrans in greater yields than from the starches.

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# ERRORS IN THE APPROXIMATIONS OF FUNCTIONS BY THE USE OF FUNCTIONALS<sup>1</sup>

ROY HERBERT COOK

*From the Department of Mathematics, Iowa State College*

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The expansion of a function in terms of sets of linearly independent functions is one to which a great deal of consideration has been given. In the main, attention has been focused on: 1. Method of expansion; 2. Convergence of the series obtained. Under 1, work has been done on the development of sets of linearly independent functions, and on the selection of sets of functionals to be used with these functions. Usually it is desirable that the set of functions and the set of functionals to be used with them form a biorthogonal sequence which is normalized to unity. Under 2, results such as the following have been the goal: Given the infinite biorthogonal sequence  $\{\Phi_i, H_i\}$  where  $\Phi_i$  is a function and  $H_i$  is a functional both defined in a region  $(a, b)$ ; one may form a sequence of sums  $\{S_k\}$  for a function  $f(x)$  as follows:

$$S_k = \sum_{i=0}^k \Phi_i H_i f(x) ,$$

and if  $f(x)$  is of a certain class, A, then

$$\lim_{k \rightarrow \infty} S_k = f(x) .$$

It is, of course, satisfying to know that each of a certain class of functions can be represented by a convergent series of terms of the above type. On the other hand, one must content himself, in practice, with finite series. Thus the question of error or remainder arises. How well does the sum  $S_n$  represent  $f(x)$ ? The present paper will discuss some aspects of this problem.

Consider the biorthogonal sequence  $\{\phi_i, F_i\}$  in terms of which any function,  $f(x)$  of a class A can be expanded so that

$$\lim_{n \rightarrow \infty} \sum_{i=0}^n \phi_i F_i f(x) = f(x) . \quad (1)$$

Further, suppose that

$$f(x) = r_n f(x) + \sum_{i=0}^{n-1} \phi_i F_i f(x) , \quad (2)$$

i. e. that a remainder operator,  $r_n$ , is known. Consider also a sequence of

<sup>1</sup>Original thesis submitted December 10, 1940. Doctoral thesis number 599.

functions  $\{\Phi_i\}$  of class B where class B is that sub-class of class A for which

$$r_k \Phi_{k-1} = 0 ; \quad (i = 1, 2, \dots, k). \quad (3)$$

Then

$$\Phi_j = \sum_{i=0}^j a_{ji} \phi_i, \quad (j = 0, 1, \dots, k); \quad (4)$$

where

$$a_{ji} = F_i \Phi_i. \quad (5)$$

The set of equations

$$\Phi_j = \sum_{i=0}^j a_{ji} \phi_i, \quad (j = 0, 1, \dots, k), \quad (6)$$

can in general be solved for the  $\phi$ 's to give

$$\phi_j = \frac{1}{\sum_{i=0}^j |a_{ij}|} A_{ti}^{-k} \Phi_i, \quad (j = 0, 1, \dots, k), \quad (7)$$

where  $A_{ti}^{-k}$  is given by

$$\frac{\text{cofactor of } a_{1t} \text{ in the determinant } |a_{11}|.}{|a_{11}|}. \quad (8)$$

Let us now form a sequence of functionals,  $\{G_i^k\}$ , as follows:

$$G_i^k = \sum_{j=0}^k A_{ji}^{-k} F_j, \quad (i = 0, 1, \dots, k). \quad (9)$$

Then:

**Theorem I.**—The sequence of functions  $\{\Phi_i\}$ ,  $(i = 0, 1, \dots, k)$  and the sequence of functionals  $\{G_i^k\}$ ,  $(i = 0, 1, \dots, k)$  form a biorthogonal sequence. That is

$$G_j^k \Phi_i = \delta_{ji} = \begin{cases} 0 & ; i \neq j \\ 1 & ; i = j. \end{cases} \quad (10)$$

**Proof:** Operate on  $\Phi_i$  with  $G_j^k$

$$G_j^k \Phi_i = \sum_{t=0}^k A_{ti}^{-k} F_t \sum_{s=0}^i a_{is} \phi_s,$$

in which  $(j = 0, 1, \dots, k)$  and  $(i = 0, 1, \dots, k)$ . Then

$$\begin{aligned} G_j^k \Phi_i &= \sum_{t=0}^k \sum_{s=0}^i A_{ti}^{-k} a_{is} F_t \phi_s \\ &= \sum_{t=0}^i A_{ti}^{-k} a_{it}, \end{aligned}$$

So  $G_j^k \Phi_i = \delta_{ji}$ ,  $(j = 0, 1, \dots, k)$ ,  $(i = 0, 1, \dots, k)$ . (11)

We may now proceed to establish a remainder operator for biorthogonal sequence  $\{\Phi_i, G_i^k\}$ . In order to do this form the operator

$$\begin{aligned}
 \sum_{i=0}^{n-1} \Phi_i G_i^k &= \sum_{i=0}^{n-1} \sum_{j=0}^i \sum_{s=0}^k a_{ij} A_{si}^k \phi_j F_s, \quad (n = 1, 2, \dots, k+1), \quad (12) \\
 &= \sum_{i=0}^{n-1} \sum_{j=0}^i \sum_{s=0}^{n-1} a_{ij} A_{si}^k \phi_j F_s, \\
 &\quad + \sum_{i=0}^{n-1} \sum_{j=0}^i \sum_{s=n}^k a_{ij} A_{si}^k \phi_j F_s, \\
 &= \sum_{i=0}^{n-1} \phi_i F_i + \sum_{i=0}^{n-1} \sum_{s=n}^k \Phi_i G_i^k \phi_s F_s, \\
 \text{thus} \quad &= \sum_{i=0}^{n-1} \phi_i F_i + \sum_{i=0}^{n-1} \Phi_i G_i^k r_n. \quad (13)
 \end{aligned}$$

Hence the remainder operator for  $\{\Phi_i, G_i^k\}$  is

$$R_n^k = r_n - \sum_{i=0}^{n-1} \Phi_i G_i^k r_n, \quad (n = 1, 2, \dots, k+1). \quad (14)$$

The limiting case in which  $k$  is allowed to become infinite is perhaps the most interesting. We shall now consider this case.

First consider the infinite biorthogonal sequence  $\{\Phi_i, H_i\}$  previously mentioned. Expand a function of class A in terms of this sequence, say  $f(x)$ .

$$f(x) = \sum_{i=0}^{\infty} \Phi_i H_i f(x). \quad (15)$$

Now allow  $k$  to become infinite in equations (6) to (14) and operate on both sides of (15) with  $G_i^{\infty}$ , and obtain

$$G_i^{\infty} f(x) = H_i f(x). \quad (16)$$

Thus a function of class A is represented by identically the same expansion in terms of both  $\{\Phi_i, G_i^{\infty}\}$  and  $\{\Phi_i, H_i\}$ . This implies that equation (14) may be written, for  $k$  infinite, as follows:

$$R_n^{\infty} = r_n - \sum_{i=0}^{n-1} \Phi_i H_i r_n. \quad (17)$$

Equation (17) provides a remainder operator in finite form. This simple form has been obtained in virtue of the restriction stated in equation (3). At first glance this restriction might seem to be rather severe; but on the contrary a large number of expansions can be dealt with by equation (17). For example if  $\{\phi_i\} = \{x_i/i!\}$  and  $\{F_i\} = \{d/dx \mid x=0\}$  we may apply (17) to such systems as Legendre polynomials, etc. This is possible because the remainder operator is known for the  $\{\phi_i, F_i\}$  just mentioned (Maclaurin's expansion).

# THE ABSORPTION OF RADIANT ENERGY IN PLANTS<sup>1</sup>

JACOB E. DINGER

*From the Department of Physics, Iowa State College*

Received June 10, 1941

A number of workers have determined the reflection, transmission, and the absorption of visible radiation by leaves, but a similar study had not been given to the near infrared region of the spectrum. In this investigation a measurement was made of the reflection and transmission by leaves of radiation in the near infrared throughout the spectral region between the wavelengths .7 microns and 2.6 microns. A determination of the scattering properties of the leaf was necessary in measuring the total transmission and reflection. A study of the absorption spectra in the visible region of extracts from the leaf was also made.

A quartz spectrometer was used to obtain a monochromatic beam of radiation. The transmitted or reflected radiation was detected and measured by means of a Coblenz thermopile in connection with a high sensitivity, low resistance Leeds and Northrup galvanometer. The thermopile, galvanometer, and a low resistance were connected in series. A circuit parallel to the low resistance made it possible to pass a current through the low resistance in a direction such that the IR drop across the resistance opposed and could be made equal to the thermopile e.m.f. as indicated by a zero deflection of the galvanometer. This current, adjusted by variable rheostats and measured by a 50 milliampere range milliammeter included in the circuit, was taken as proportional to the radiation reaching the thermopile. Closing a switch opened an electromagnetic light shutter which permitted radiation to reach the thermopile and simultaneously closed the circuit setting up the current in the thermopile circuit which produced the IR drop opposing the thermopile e.m.f.; in this way a null method of taking readings was achieved. A galvanometer deflection amplifier was used to determine very accurately the zero deflection of the galvanometer. A beam of light was reflected from the mirror of the galvanometer and focused on a right angle mirror placed between two phototubes. The light was in this way reflected on either one or both of the phototubes depending upon the position of the galvanometer mirror. The output of each phototube was amplified by a two-stage d.c. amplifier, and was imposed on a double target electric eye tube so that with equal intensities of light on the two phototubes, a symmetrical pattern was formed on the electric eye tube. A very slight deflection of the galvanometer caused a relative change in the light intensities reaching the phototubes which resulted in a change in

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the electric eye tube pattern. This arrangement made it possible to detect a change of voltage of the order of  $5 \times 10^{-9}$  volts.

The percentage transmission and reflection of the leaf was obtained by comparing the leaf transmission and reflection with that of bond paper which had very similar scattering properties. By choosing a grade of paper with scattering properties very similar to the leaf and having a known reflection and transmission factor, the ratio of the reading taken with a leaf to the reading taken with the paper multiplied by the reflection or transmission factor gave the total reflection or transmission of the leaf. Readings were taken at intervals of .1 micron with a spectral purity of .1 micron or less. The reflection and transmission was relatively high in the region from .8 micron to 1.3 microns, there being approximately 15 per cent absorbed. The water bands at 1.5 microns and 2.0 microns were very pronounced in the reflected as well as the transmitted radiation, which indicated that a large portion of the reflected radiation was internally reflected.

The absorption spectra of acetone-ether extract, acetone-petroleum ether extract, yellow pigments in ether, and saponified chlorophyll in water were obtained for a series of concentrations. Readings were taken throughout the visible region from .35 to .7 microns at intervals of .025 micron and with spectral purity of .020 microns. Beer's Law can be given by the expression  $I = I_0 e^{-c\beta d}$  where  $I_0$ ,  $I$ ,  $c$ ,  $\beta$ , and  $d$  are the incident radiation, transmitted radiation, concentration, absorption coefficient for unit thickness and concentration, and the thickness respectively. It was found that Beer's Law is valid for the above solutions having concentrations less than .03 gm./liter, but for greater concentrations there a very marked deviation in the regions of high absorption especially in the short wavelength end of the visible spectrum. By taking the difference between the absorption coefficients of the acetone-ether extract, which contained the yellow pigments plus chlorophyll, and the yellow pigments in ether solution, the absorption spectra for the chlorophyll alone was obtained.

No absorption was observed in the near infrared region from .75 to 2.6 microns.

MEAT IN NUTRITION. XVIII. GLYCOGEN IN MATERNAL AND  
FETAL LIVERS OF RATS FED A DIET CONTAINING DRIED  
AUTOCLAVED PORK MUSCLE<sup>1</sup>

HELEN ELIZABETH FARRANKOP

From the Department of Foods and Nutrition, Iowa State College

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The study to be reported is one in a series of investigations in progress in the Foods and Nutrition Laboratory of Iowa State College relating to the effect of feeding a diet containing pork muscle upon the reproductive performance of the albino rat. The experimental diet contained dried, canned pork muscle as its source of protein. Gestational failure and a so-called "toxemia" of pregnancy are frequent occurrences in female rats reared upon this diet. Histological examinations and chemical analyses have shown that fatty infiltration and degeneration of the liver are characteristic of the disease. It has been suggested that a reciprocal relationship exists between the amount of fat and glycogen in the liver. The present study was undertaken to test the hypothesis that a derangement of carbohydrate metabolism may occur in the rats fed the pork diet. Animals reared upon the stock ration, a whole grain-casein diet designated as Steenbock V, served as controls. The experimental diet was called Pork I. The concentration of glycogen present in maternal and fetal livers was used as an index of the status of carbohydrate metabolism.

In the first series of experiments, the content of glycogen in the livers of pregnant control rats was compared with that of virgins of the same age reared on the same diets. Subsequent to a 13-hour fast, all the animals were fed 4 gm. of the diet upon which they had been maintained. After 7 hours, the rats were stunned by a blow on the head and the liver quickly removed for analysis. Pregnant animals were killed on the 21.5 day of gestation and virgins when they were approximately the same age as the gravid rats. The livers were dropped into tared tubes of potassium hydroxide. The quantity of glycogen was determined by the Good-Kramer modification of the Pflüger method. The Shaffer-Somogyi procedure for the analysis of glucose was used.

Both the absolute and relative amounts of glycogen present in the livers of the pregnant control animals were significantly higher than those in the livers of the pregnant pork-fed group. The average percentage composition of glycogen was 3.18 for the controls, and 2.57 for the experimental rats.

In each group the relative concentration of glycogen was higher in the livers of virgins than in the livers of pregnant animals (4.08 vs. 2.57 per cent respectively in pork-fed rats and 3.58 vs. 3.18 per cent in the control

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rats). In the virgins fed the Steenbock diet, the absolute amount of glycogen was 213.1 mg. compared with 246.2 mg. in the livers of the pregnant animals. In the pork-fed virgins it was 231.7 mg., and in the pork-fed pregnant rats, 193.2 mg. In both groups, the livers of virgin animals were lighter than those of the pregnant rats, and the difference was highly significant. It appears, therefore, that the effect of pregnancy in rats is to lower the relative amount of glycogen present in the liver, and that this decrease is greater in the case of the animals fed the pork diet than in the animals reared on the stock ration. Pregnancy also results in an increase in the size of the liver.

The pregnant rats reared on the pork ration were next sorted into two groups, composed of those with total resorptions and those with living feti. Comparisons were made of absolute and relative amounts of glycogen in the livers of these two groups to determine whether the lowering of glycogen already noted in the pork-fed animals could be attributed to the frequent occurrence of total resorptions. It was found that total resorptions account for only a part of the fall in glycogen concentration observed in the livers of the experimental animals maintained on the pork diet. Neither did the quality of the test meal influence glycogen storage in the livers of the pregnant experimental rats.

Fetal livers were removed from part of the pregnant rats belonging to the first two series. The relative amounts of glycogen in the fetal livers from the two groups (6.10 vs. 5.92 per cent) did not differ significantly, but the average weight of fetal livers per litter was significantly higher in the control rats (1.9994 gm.) than in the pork-fed rats (1.6505 gm.). Thus, it seems that mothers reared on the stock diet were able to produce relatively more fetal hepatic tissue and to store more glycogen in the fetal livers than were the experimental rats.

Females from the two groups were next matched according to the number of feti present in the uterus and the relative concentration of glycogen examined. In almost all cases, the control rat contained a higher percentage of maternal liver glycogen than did the pork-fed rat with the corresponding number of feti. In the control group the percentage of fetal glycogen increased as the number of feti decreased, but the concentration of maternal glycogen remained fairly consistent. The evidence accumulated points to the conclusion that production of glycogen is definitely decreased in pregnancy by feeding the diet containing pork muscle.

In the series of experiments, in which a standard quantity of glucose was administered by stomach tube after starvation, the data showed that there was no abnormality in intestinal absorption in the pork-fed rats, and that the glycogen stores of the livers were largely depleted during the 13-hour starvation period. Neither could any difference be observed in the absolute and relative amount of glycogen found in the livers of the pregnant controls (45.3 mg. and 0.66 per cent) and in the livers of the pregnant experimental rats (46.8 mg. and 0.64 per cent) 4 hours after

the feeding of the glucose solution. In both groups, the livers of the virgins were lighter than those of the pregnant animals, and contained a higher absolute and relative quantity of glycogen. In this series, as in the first, there was a greater quantity of glycogen in the liver of the virgin rats reared on the pork diet than in those of the virgin rats reared on the stock diet. Again, it may be concluded that pregnancy is associated with a decreased percentage of liver glycogen. Also, in the pork-fed rats there was a greater difference between the level of glycogen stored in the livers of virgin and pregnant animals than there was in the Steenbock-fed animals.

A glycogen index was calculated for each rat in the third series by using the formula:

$$\frac{\text{glycogen (mg. per 100 gm. of hepatic tissue)}}{\text{mg. sugar absorbed per 100 gm. of body wt.}}$$

Glycogen indices thus obtained again showed that pregnancy reduces the stores of glycogen and that no difference exists between experimental and control animals.

Data pertaining to the fourth series consisting of pregnant pork-fed rats killed on the twenty-second day of gestation showed that no further breakdown of carbohydrate metabolism occurred on the last day of gestation in the experimental rats.

The results of the determinations for the third and fourth series yielded little information as to the status of carbohydrate metabolism in the pregnant pork-fed animals because the values obtained for glycogen so nearly approached the starvation level. In any further investigation of this problem, it is recommended that larger quantities of glucose be administered and that the absorption period be shortened, especially in working with pregnant animals.

The two animals of the fifth series that exhibited typical "toxic" symptoms showed no liver glycogen. It is believed that the derangement of carbohydrate metabolism noted in all pregnant rats reared on the pork diet on the twenty-first day of pregnancy becomes acute whenever an eclamptic condition develops.

# AN ANALYSIS OF GROUP DIFFERENCES ARISING FROM A POISSON DISTRIBUTION OF OBSERVATIONS OBTAINED FROM IRRADIATION EXPERIMENTS<sup>1</sup>

HOLLY CLAIR FRYER

*From the Department of Mathematics, Iowa State College*

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It is well-known that the chromatin of germ-cells is subject to spontaneous alterations. These may involve large sections of the chromosomes or they may be localized about a particular gene. This latter type of change offers a means of investigating the nature and actions of the gene. Since any data collected on these gene changes are subject to both random and non-random variations, the statistical analysis becomes of major importance in any interpretation.

After irradiation experiments had shown that changes in the chromatin could be produced artificially, similar statistical problems arose in the analysis of such mutation data.

When a set of observations is regarded as constituting a sample from a population of like data, the sampling distributions characteristic of this population must be considered. Irradiation data fit a binomial type of distribution. The probability function for such a distribution is the familiar one,

$$\frac{n!}{(n-r)! r!} p^r (1-p)^{n-r},$$

where  $p$  = the probability of a mutation at a particular locus,  
 $r$  = the number of mutations observed at that locus, and  
 $n$  = the number of times that gene-locus was observed.

Since particular mutations occur infrequently, the probabilities involved in the distributions are quite small, usually lying within the range  $.001 \leq p \leq .05$ . For such small probabilities, the corresponding binomial distributions are not well-approximated by normal distributions, but are by appropriate Poisson distributions. Consequently, irradiation data are said to be distributed in a Poisson manner.

The possible sources of non-random variation among irradiation data are suggested either by biological and physical theory or by the data themselves. One of the classical methods for measuring the randomness of the "residual" variation about the basic hypotheses adopted is the chi-square test. It is well-known that for sufficiently large expected values,  $\bar{x}$ , the function

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$$\frac{\sum_{i=1}^N (x_i - \bar{x})^2}{\bar{x} (1 - \frac{\bar{x}}{n})},$$

where  $x_i$  = observed number in the  $i$ th class,

$$\bar{x} = \frac{\sum x_i}{Nn},$$

$N$  = number of classes, and

$n$  = number of observations on each class,

is distributed approximately as a Pearsonian Type III curve. The probability integrals of this curve are tabulated in the usual chi-square tables. We use these tables for convenience if we believe that they give close approximations to the exact chi-square probabilities.

Until recently the closeness of this approximation was in doubt for expectations even so large as 10 or 15; but some research by Neyman and Pearson, Sukhatme, Cochran, and Hoel has shown that the agreement between the exact and the tabular probabilities is surprisingly good when expectations are as low as 2.

Since much mutation data involves expected values less than 2, it was thought necessary to investigate the goodness of the Type III approximation for expectations lower than 2. For small numbers of observations, the exact distribution of chi-square can be obtained without difficulty. The exact probability associated with any chi-square in the set can then be obtained and compared with the corresponding tabular probability. This was done for a large number of samples with expectations ranging between 0.5 and 1.5, illustrated by irradiation data. It was found that the agreement between the two probabilities was entirely satisfactory for a test of significance when the exact probabilities were less than 0.100. This is the usual region of interest in such tests.

Since Fisher is of the opinion that the likelihood function,  $L$ , is our best measure of the agreement between observation and expectation, the exact likelihood and the exact chi-square probabilities were compared for the same ranges of expectation mentioned above. It was found that in not over 10 per cent of the samples studied were the conclusions altered by using one test instead of the other. Thus chi-square derives added justification from its agreement with  $L$ .

New irradiation data were collected in the course of these investigations to furnish independent tests of, and illustrative material for, the problems of mutation in living things. Nineteen different loci on the first three chromosomes of *Drosophila melanogaster* were exposed to x-rays of three different wave-lengths and at two intensities. These new data

were analysed by the methods developed in this study and led to the following conclusions: (1) the data give no reason to doubt the following three hypotheses: (a) that mutation rate at a specific locus is directly proportional to the dosage of radiation applied, (b) that if the sets of genes used are representative of their respective chromosomes, the fundamental rates of mutation on the first three chromosomes of *Drosophila melanogaster* are the same, and (c) that for a particular gene and a fixed dosage, the wave-length of the x-rays does not affect the mutation rate; but (2) the hypothesis that the genes within the sets observed have the same basic mutation rate is inadequate to explain the variation observed when the "ct" locus is among those considered. This gene, when contrasted with the other eighteen, apparently has a higher rate of change.

Other sets of data besides our own were analysed by the chi-square test as illustrations of methodology. From these tests the following conclusions were drawn: (1) there is little reason to believe that, for a fixed dosage, interruption of the irradiation affects the mutation rates; (2) there is no evidence in the data analysed to indicate that there are different rates of reverse mutation among the genes observed; (3) high temperatures may increase the lethal mutation rate if the male is the sex treated, but there is no evidence that keeping the female at high temperatures will produce mutations; and (4) there is no evidence in the data considered to indicate that different species of drosophila have basically different rates of lethal mutation.

The analyses of the biological data within the scope of this study in conjunction with the mathematical examination of comparable chi-square distributions justify the extension of this method to tests of significance of irradiation data.

# THE POLAROGRAPHIC METHOD IN THE INVESTIGATION OF SEVERAL ACETOBACTER SUBOXYDANS FERMENTATION PRODUCTS<sup>1</sup>

EAKIN M. GLYMPH

From the Department of Chemistry, Iowa State College

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*Acetobacter suboxydans* is noted for its mild oxidative action upon polyhydric alcohols resulting, for the greater part, in a simple dehydrogenation of the alcohol to a ketone. Furthermore, the dehydrogenating action of this organism is highly specific for certain structural configurations of the molecule attacked.

In this thesis, the polarographic method has been employed in the investigation of a series of ketones formed by the action of *Acetobacter suboxydans*. The series included dihydroxyacetone, erythrulose, sorbose, tagatose, and a "ketose" produced from *i*-inositol. Dunning, Fulmer, Guymon, and Underkofler (3) have shown "ketose" to be principally a diketo-*i*-inositol. The dehydrogenation of *d*-talitol to form tagatose by the action of *Acetobacter suboxydans* has not been reported, probably because of the scarcity of *d*-talitol. However, since this polyhydric alcohol has the proper configuration for fermentation, the appearance of tagatose in the above series is justifiable. The reductions performed at the dropping mercury cathode were essentially the reverse processes of those performed by the organism *in vivo*.

The current-voltage data were obtained by the manual method. The current was determined by measuring the *IR* drop across a standard resistance and applying Ohm's law. The half-wave potentials for the reductions of the compounds in 0.1 M lithium chloride, referred to the saturated calomel half-cell and arranged in order of increasing negative potential, are as follows: "ketose", -1.55 volts; dihydroxyacetone, -1.59 volts; erythrulose, -1.60 volts (a second small "wave" was observed between -1.80 and -1.90 volts); sorbose, -1.81 volts; and tagatose, -1.81 volts. The half-wave potential of sorbose agrees favorably with the value found by Heyrovský and Smoleř (1). Considering the compounds investigated in this work along with other compounds formed by the action of *Acetobacter suboxydans* which have been investigated polarographically (such as fructose, galactose, acetaldehyde, propionaldehyde, acetone, hydroxyacetone, acetyl methyl carbinol, and diacetyl), there appears to be no direct relationship between the polarographic half-wave potential of the compound and the ability of the organism to form the compound from the corresponding alcohol.

If the above-listed polarographic half-wave potentials have any

<sup>1</sup>Original thesis submitted June, 1941. Doctoral thesis number 619.

thermodynamic significance, then "ketose," the oxidant of a more positive system, should oxidize sorbitol, the reductant of a less positive system, provided a suitable catalyst is present. Palladium black and a bacterial juice prepared from *Acetobacter suboxydans* were employed in an attempt to equilibrate the reaction between "ketose" and sorbitol. Neither substance showed catalytic activity. The bacterial juice also was used as a "potential mediator" in the reduction of dihydroxyacetone, but no appreciable effect on the half-wave potential was observed. However, it is suggested that the polarographic method may be employed successfully in certain enzyme-catalyzed equilibrium studies.

The effect of pH on the half-wave potentials of dihydroxyacetone, sorbose, and "ketose" has been found to be less than expected from theoretical considerations. The reductions are irreversible, although in the case of "ketose" there might exist a reversible step, the nature of which is at present unknown. The  $E_o$  for the sorbose-sorbitol system calculated from thermal data does not agree with the half-wave potential for the reduction of sorbose (calculated for pH = 0). The calculation of this  $E_o$  was based on some reasonable assumptions. Evidence that the equation of the polarographic "wave" does not hold in the reduction of sorbose and "ketose" is given by the fact that the straight lines obtained by plotting  $E_c$  against  $\log (I/I_d - I)$  do not have slopes equal to 2.303 ( $RT/nF$ ). The slope in the case of sorbose was 0.142 while that for "ketose" was 0.083 as compared to theoretical values of 0.0295 and 0.0148 respectively.

The diffusion currents observed for the different compounds studied have been found to be less than those calculated by use of the Ilković equation (Ilković, 2) except in the case of erythrulose. The reductions of erythrulose were carried out in diluted fermentation liquors instead of in pure solutions; therefore, the data are probably less accurate. Explanations for the above-mentioned discrepancies have been presented based on the existence of isomeric forms of the compounds in solution. Heyrovský and Smolef (1) previously have attributed the failure of certain sugars to be reduced completely at the dropping mercury cathode to the existence of difficultly reducible isomeric forms.

In buffered basic solutions (borate and carbonate buffers at a pH of about 10) the diffusion current of dihydroxyacetone has been observed to decrease with time. After standing for a period of less than a day out of contact with air, this compound showed no reduction "wave." This phenomenon was possibly due to conversion of the easily reducible keto form of dihydroxyacetone to the more difficultly reducible enol form. Supporting evidence that the enol form of dihydroxyacetone is a difficultly reducible form is given by the fact that ascorbic acid, a compound having a double-bond structure very similar to that in the enol form of dihydroxyacetone, is not reducible at the dropping mercury cathode. The diffusion current of "ketose" also has been observed to decrease with time when the compound is present in strongly basic

solutions. Furthermore, "ketose" developed a bright yellow color after standing several hours in a carbonate-bicarbonate buffer (pH=9.8). The yellow color disappeared immediately when the solution came in contact with air, and a reduction "wave" was no longer observed.

"Ketose" has been found not to give two separate reduction "waves" as is characteristic of several diketones; however, the reduction "wave" for two ketonic groups could easily overlap. At equimolar concentrations the diffusion current for "ketose" was approximately twice as great as that for sorbose, which might possibly indicate a diketone in the former case.

The reduction of "ketose" in 0.1 M potassium chloride gives a steep maximum on the current-voltage curve. This "ketose" maximum was also observed in several of the buffers used but not in 0.1 M lithium chloride.

The diffusion currents for sorbose and "ketose" have been shown to be strictly proportional to the concentration of these compounds. Furthermore, "ketose" and sorbose may be analyzed quantitatively in the same solution. Dihydroxyacetone, erythrulose, and "ketose" may be detected in diluted fermentation liquors without the removal of proteins. The use of the polarographic method in following the course of formation of reducible materials in a fermentation has been demonstrated.

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# MINERAL ACIDS AND MOLD AMYLASE AS SACCHARIFYING AGENTS FOR PRODUCTION OF FERMENTABLE SUGARS FROM STARCH<sup>1</sup>

KENNETH JUSTIN GOERING

From the Department of Chemistry, Iowa State College

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## INTRODUCTION

Before corn or any other starchy material can be fermented by yeast, the starch must be converted into fermentable sugars. This process is called saccharification. In the conventional process malt is used for this purpose. Since malt is rather expensive, a cheaper method of saccharification is desirable if corn is to compete with molasses for ethanol production. The following investigation was undertaken with this purpose in mind.

## METHODS

Various mineral acids at different concentrations were used for the conversion of corn meal and cornstarch into fermentable sugars. The influence of mash concentration, time of heating, and steam pressure used were studied. After finding the optimum conditions for cornstarch, the same conditions were used for the saccharification of cassava starch. The use of a mold amylase preparation, produced by growing a strain of *Aspergillus oryzae* on wheat bran, both alone and in conjunction with acid hydrolysis was also investigated. The efficiency of the saccharification was determined by fermenting the mashes with yeast. The ethanol content was determined in each case, when the fermentation was complete, by distilling the fermented mash and collecting the first 100 ml. of distillate. The specific gravity ( $25^{\circ}/25^{\circ}$ ) of the distillate was measured and the ethanol content read from a table. The ethanol yields obtained were calculated as percentage of that theoretically obtainable from the starch originally present.

## RESULTS

Acid saccharification of corn meal using 0.10 normal hydrochloric acid and a steam pressure of 30 pounds for 3 hours produced an ethanol yield of 74.8 per cent of theoretical, which was equivalent to that obtained by the conventional process using 10 per cent malt. In general, higher steam pressures and shorter periods of heating produced the greatest concentration of fermentable sugars when corn meal was hydrolyzed by acid.

Cornstarch was found to be a better substrate than corn meal for the

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production of ethanol. The yields of ethanol from corn meal and corn-starch hydrolyzed with 0.10 normal sulfuric acid and fermented under the same conditions were respectively 68.6 and 88.3 per cent of theoretical. The lower yields obtained from corn meal were found due to the presence of corn bran, which when hydrolyzed apparently produced something toxic to yeast.

Cornstarch was found to be readily saccharified by dilute mineral acids. The yield of ethanol reached 100 per cent of theoretical when 7.5 per cent starch mashes were hydrolyzed with 0.02 normal sulfuric acid at a steam pressure of 25 pounds for 4 hours. The ethanol yield obtained from acid-saccharified cornstarch was a function of mash concentration, acid concentration, kind of acid employed, steam pressure, and time of hydrolysis.

Cassava starch was found to be more difficult to saccharify than corn starch. When a 10 per cent cassava mash was hydrolyzed for 4 hours at 20 pounds steam pressure with 0.20 normal sulfuric acid, the ethanol yield obtained was 82.1 per cent of theoretical.

The addition of small amounts of mold-bran greatly increased the yield of ethanol obtained from starch mashes partially saccharified by dilute mineral acids. The addition of 4 per cent mold-bran to 7.5 per cent starch mashes saccharified with 0.05 normal sulfuric acid at a steam pressure of 15 pounds for 4 hours increased the ethanol yield from 89.4 to 96.0 per cent of theoretical. When 16 per cent mashes were hydrolyzed under similar conditions, the addition of 4 per cent mold-bran increased the ethanol yield from 78.9 to 87.8 per cent of theoretical. The addition of larger amounts of mold-bran to either 7.5 or 16 per cent mashes did not increase the ethanol yield obtained to any appreciable extent. The addition of malt to acid-saccharified mashes did not increase the ethanol yield obtained. Mold-bran produced higher yields of ethanol from cornstarch than it did from corn meal. At the optimum concentration of 15 per cent mold-bran, an ethanol yield of 92.2 per cent theoretical was obtained from 16 per cent starch mashes. The use of 6 per cent mold-bran produced an ethanol yield of 86.7 per cent of theoretical. Corn-starch also proved to be a better substrate than corn meal for the production of ethanol when mold-bran was used for saccharification. The yields of ethanol from corn meal and cornstarch saccharified with 10 per cent mold-bran were, respectively, 79.8 and 88.5 per cent of theoretical.

Acid saccharification under optimum conditions, 4 per cent mold-bran in conjunction with acid, and 8 per cent mold-bran alone produced ethanol yields of 86.3, 87.8, and 87.2 per cent of theoretical, respectively, when used on 16 per cent starch mashes. Therefore, any one of the three methods may be considered satisfactory. On 7.5 per cent starch mashes, acid saccharification is the most satisfactory of these three methods.

# EFFECTS OF pH AND OF VARIOUS CONCENTRATIONS OF SODIUM, POTASSIUM, AND CALCIUM CHLORIDE ON MOTILITY OF THE ISOLATED CROP OF PERiplaneta americana<sup>1</sup>

JAMES T. GRIFFITHS, JR.

From the Department of Zoology and Entomology, Iowa State College

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In order to determine an optimum salt mixture which would serve as a suitable environment for excised tissue from the cockroach, *Periplaneta americana* L., investigations were conducted which used the isolated crop of the American roach as the test tissue. A method for removing the foregut and for recording its muscular activity was described, and the latter was diagrammed. An activity product (average amplitude in centimeters multiplied by the longevity of the tissue in hours) was used as a criterion of the adequacy of a given solution. Five crops from males and five from females were tested in each solution. Sodium chloride, calcium chloride, potassium chloride, sodium bicarbonate, and sodium dihydrogen phosphate were the inorganic constituents of the various solutions. Eighty-three different mixtures were tried.

The literature concerned with the use of physiological solutions as media for the study of isolated insect tissues was reviewed. A brief discussion of the effects of changing the concentrations of either sodium, calcium, or potassium was included.

In order to establish a base for further experimentation, the first solutions which were tested contained 1.0 per cent sodium chloride, 0.02 per cent sodium bicarbonate, varying amounts of potassium chloride (0.0, 0.2, 0.4, 0.6, and 0.8 gram per liter of solution) and calcium chloride (0.0, 0.2, 0.4, 0.6, and 0.8 gram per liter). These 25 mixtures had pH values between 7.5 and 8.0. It was found that seven potassium chloride/calcium chloride ratios at the 1.0 per cent sodium chloride level produced decidedly superior records. These ratios were 0.2/0.2, 0.4/0.4, 0.6/0.6, 0.8/0.8, 0.2/0.4, 0.4/0.6, and 0.6/0.8, expressed as gram of potassium chloride to gram of calcium chloride per liter. The seven solutions with the selected ratios had average activity products which varied between 0.390 and 0.549. The remaining 18 mixtures have average activity products which were less than 0.310 in every instance. Thus, it was evident that these seven selected ratios were considerably better than the others which were tested. The selected ratios were within the range of 0.5 to 1.0.

It was demonstrated that potassium in the absence of calcium caused an immediate loss of tone and arrest in relaxation. Likewise, in more than 50 per cent of the cases, calcium in the absence of potassium caused an initial tone increase and arrest in rigor.

<sup>1</sup>Original thesis submitted June, 1941. Doctoral thesis number 615.

Several sodium chloride concentrations (12.0, 13.0, 14.0, 15.0, 16.0, 17.0, and 18.0 grams per liter of solution) were tested with the seven selected potassium chloride/calcium chloride ratios. This was done in order to ascertain the effect of increased sodium and also to determine an optimum sodium chloride concentration. The pH of these 49 mixtures was maintained between 7.5 and 8.0 by the addition of 0.2 grams of sodium bicarbonate per liter. The average activity products of the eight sodium chloride levels could be compared. Thus, it was found that there was essentially no difference in activity between the 1.0, the 1.2, and the 1.3 per cent levels. The average activity products for the 1.4 per cent sodium chloride mixtures showed a very marked increase. At this level, the highest average activity product, 0.795, was obtained. High activity products were also produced in the 1.5 and 1.6 per cent levels. The average activity products were considerably decreased in 1.7 per cent sodium chloride and were further decreased in 1.8 per cent. It seemed that 1.4 per cent sodium chloride offered an optimum condition for the activity of the isolated foregut of the American roach. Apparently, the crop could accommodate itself to large shifts in the sodium chloride concentration and, also, to considerable change in the osmotic pressure.

The optimum ratio of potassium chloride/calcium chloride could be determined by examination of the results from this last series of experiments. The ratios which were less than one produced greater average activity products than those which were equal to one. In addition, as the actual amounts of potassium and calcium were increased, the activity of the crops was decreased. Thus, better activity was obtained in a ratio of 0.02 per cent/0.02 per cent than in a ratio of 0.08 per cent/0.08 per cent; similarly, better activity was obtained in a ratio of 0.02 per cent/0.04 per cent than in one of 0.06 per cent/0.08 per cent. The optimum potassium chloride/calcium chloride ratio was 0.2/0.4 gram per liter.

In 1915, Glaser had stated that the pH of the hemolymph of the American roach was 7.5 to 8.0. All of the test solutions were made up within this pH range. Later, values of 6.8 and 8.9 were also used in order to study the effect of a shift in pH. Four mixtures were tried at each pH value. These solutions contained 1.4 per cent sodium chloride and potassium chloride/calcium chloride ratios of 0.2/0.2, 0.4/0.4, 0.2/0.4, and 0.4/0.6 gram per liter. At 6.8 the pH was adjusted by the use of sodium dihydrogen phosphate and titration with tenth normal sodium hydroxide. At this pH value, activity was considerably reduced over that at 7.8. Sodium bicarbonate was titrated with tenth normal sodium hydroxide to obtain the pH of 8.9. Although the average activity product at this pH was better than at 6.8, it was definitely lower than at 7.8. It appears, therefore, that a value which is between 7.5 and 8.0 is the optimum condition for activity of the isolated crop of the American roach.

It was noted that the activity products for crops from females were usually less than those for crops from males. The average activity product for the males' crops was 0.525, and for the females', 0.399. In 70 per cent of the solutions the crops from males had higher average activity products. In spite of the fact that both males and females had access to food at the same time, it was found that when the foreguts were isolated, those from females contained more food than those from males. Additional experimentation showed that there was not a significant difference in the amount of food ingested by the males and females. Apparently, food passes more slowly through the foregut of the female than of the male. Thus, it appeared that the difference in activity of the excised crops was due to an inherently greater contraction rate in the crop of the male.

As a result of these investigations, it was concluded that, of the salt concentrations tested, the optimum mixture was one which contained 14.0 grams of sodium chloride, 0.2 gram of potassium chloride, 0.4 gram of calcium chloride, and 0.2 gram of sodium bicarbonate per liter of solution. This mixture would have a pH value of 7.5 to 8.0.

# EFFECTS OF DIETARY MODIFICATIONS ON THE TAPEWORM HYMENOLEPIS DIMINUTA<sup>1,2</sup>

ANNA HAGER

From the Department of Zoology and Entomology, Iowa State College

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White rats of the Wistar A inbred strain were experimentally infected with the rat tapeworm *Hymenolepis diminuta* by means of confused flour beetles (*Tribolium confusum*) as vectors. The beetles were fed gravid proglottids from the posterior portions of rat tapeworms, and, after three weeks to allow for the development of cysticercoids, were decapitated and added to the food of the rats. Those rats which became infected began to pass worm eggs in approximately three weeks after ingestion of the intermediate hosts.

The rats were kept in separate cages of half-inch mesh hardware cloth beneath which were placed enameled pans containing water and a small amount of trisodium phosphate. The pellets dropped through the mesh floors and were caught in the pans below. Every other day each pan was removed and replaced by a clean one, and the contents, including all of the worm eggs egested during the preceding two days, were thoroughly mixed and diluted to one liter.

Of this suspension, made as homogeneous as possible, a small amount was dropped into a mold counting chamber of known volume. The eggs present in this volume were determined, and from this count the approximate number present in the liter of material, and the numbers eliminated per day per rat, were calculated. Rats were weighed each week.

For a month after the first appearance of eggs in the feces, all rats in any experiment were given the same control diet,<sup>3</sup> after which a part of the hosts were given the experimental diet to be used in that investigation. In this way, the numbers of eggs eliminated by each rat on the experimental diet could be compared with its own record on the control diet, and groups of rats could be compared while receiving similar and dissimilar diets.

There was a considerable day-to-day variation in the numbers of eggs present in the fecal material. It was necessary, therefore, to consider the general trend of the counts rather than to attempt an explanation for each rise or fall. The egg counts for one rat could not be compared directly with those for another, since there was no assurance that they were harboring the same number of worms.

<sup>1</sup>Original thesis submitted June 3, 1939. Doctoral thesis number 513.

<sup>2</sup>The entire paper based upon this thesis was published in the Iowa State College Jour. of Sci. 15: 127-154, 1941.

<sup>3</sup>Cellufibre, 3 per cent; beet sugar, 63 per cent; casein, 15 per cent; complete salt mixture, 4 per cent; cod liver oil, 2 per cent; lard, 3 per cent; Fleischmann's yeast, 10 per cent.

At the conclusion of each experiment, the hosts were killed with illuminating gas and the cestodes removed, counted, and measured.

### RESULTS

1. Rats transferred to an exclusive whole milk diet eliminated consistently fewer tapeworm eggs than they had passed while on the control diet; whereas the numbers of eggs eliminated by control rats, not shifted from the control diet, did not change significantly. Eight rats were studied over a period of five months.

2. Two young rats were transferred from the control ration to one in which 10 per cent more sugar was substituted for the 10 per cent yeast. This diet was inadequate for vitamins B<sub>1</sub> and G. As compared to the two controls, these rats declined rapidly in weight and health, and the numbers of worm eggs also declined, disappearing from the droppings of one. The numbers of eggs mounted again when the hosts were restored to the control diet.

3. Yeast autoclaved for 2 hours at 120° C. was substituted for unautoclaved yeast to supply a ration deficient in vitamin B<sub>1</sub>. Rats transferred to this inadequate diet ceased to gain and after several weeks fell off sharply in body weight, but there was no marked decrease in the numbers of worm eggs eliminated. Ten rats were used in this investigation.

4. Four young rats were given a ration similar to that used to produce a deficiency of vitamin B<sub>1</sub> and G, but vitamin B<sub>1</sub> was added by the daily administration of four drops of tiki tiki. As compared to the four control animals, those deficient in vitamin G lost weight although they continued to eat fairly well. The numbers of eggs passed were greatly decreased. Restored to the control diet, the experimental animals regained weight and an appearance of health and tidiness, and the reproductive of the cestode parasites also increased.

5. Six young rats were maintained on the control diet, but were allowed only one-third as much food as the average amount eaten by six controls. After two months, the groups were reversed in treatment. In neither group did the numbers of tapeworm eggs eliminated decrease appreciably.

6. Rats given a diet in which soybean oil meal was substituted for yeast, eliminated fewer worm eggs than those on a diet in which wheat middlings took the place of the yeast and a part of the sugar. When the groups were reversed in treatment, soybean oil meal continued to be associated with lower egg counts than did wheat middlings.

7. Regardless of host diet prior to death, an inverse relationship was observed between the numbers of worms harbored by any one host and their average length.

8. During the periods when all the rats received the control diet, the average numbers of eggs produced per worm varied inversely with the worm burden.

## CONCLUSIONS

The adult flour beetle *Tribolium confusum* may serve as a vector for the rat tapeworm *Hymenolepis diminuta*. The cysticercoids may infect the rat after approximately three weeks.

There is no direct relationship between the worm burden and the numbers of eggs eliminated by the host, but rather an inverse relationship between the worm burden and the lengths and reproductive powers of the parasites harbored.

A diet deficient in vitamins B<sub>1</sub> and G is associated with a decrease in egg output. This effect is apparently not due to the labile vitamin B<sub>1</sub>. There appears to be a factor associated with the vitamin G complex which is necessary for normal egg production by the rat tapeworm. A diet lacking in vitamin G does not furnish this factor. It is not maintained that the factor involved is identical with vitamin G. A diet containing wheat middlings is associated with a higher production of tapeworm eggs than is one containing soybean oil meal. This may be attributable to a larger amount in the former of the factor necessary for reproduction.

A diet consisting exclusively of whole milk is associated with a decrease in the numbers of eggs developed.

Partial starvation of the host by restriction of the food intake to one-third the normal amount does not cause a decrease in the numbers of tapeworm eggs eliminated.

# THE EFFECT OF CONTROLLED pH UPON THE PRODUCTION OF CHEMICALS IN SEVERAL FERMENTATIONS<sup>1</sup>

RICHARD JAMES HICKEY

From the Department of Chemistry, Iowa State College

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## INTRODUCTION

Most of the glycerol produced in the United States at present is a by-product of the soap industry. Recently a method of synthesis from propylene has been described by Williams and associates (8) (9). Methods for producing glycerol by fermentation have been suggested—Connstein and Lüdecke (2) (3), Cocking and Lilly (1), and Eoff, Linder, and Beyer (4)—but have not proven economically successful on a commercial basis in normal times. These procedures have utilized sodium sulfite, a mixture of sodium sulfite and bisulfite, or sodium carbonate. Considerable difficulty was encountered in recovering the glycerol from the fermented mashes containing the high proportions of sodium salts required. However, fermentation glycerol was produced as a military necessity at a rather high cost in Germany during the World War of 1914-1918.

This thesis was concerned principally with the effect of pH on the fermentative production of glycerol by both the alkaline and sulfite mechanisms postulated by Neuberg and co-workers (7).

## METHODS

A Cameron pH Recorder was adapted for automatic pH control by adding electrical circuits and equipment. This apparatus permitted controlling the pH during fermentation, by automatic addition of base or acid, to within  $\pm 0.05$  unit of the desired pH value. A semisynthetic medium was employed which contained dextrose and a minimum of dissolved materials other than sugar in order to simplify analyses. The rapid and accurate semimicro method for determination of glycerol described by Fulmer, Hickey, and Underkofler (5) was used. The yeast culture employed was a strain of *Saccharomyces cerevisiae* designated in these laboratories as No. 43.

## RESULTS

Krug and McDermott (6) used ammonium hydroxide to increase glycerol production by yeast fermentations. Studies of fermentative glycerol production were attempted at controlled pH levels employing ammonium hydroxide as the alkalinizing agent. The activity of the yeast

<sup>1</sup> Original thesis submitted June, 1941. Doctoral thesis number 630.

was inhibited in all cases when the pH was maintained in the alkaline range by the above reagent. Studies were made on the fermentability of the medium as a function of the ammonium concentration. At pH = 6.5, with the ammonium concentration at 0.1 to 0.5 normal, the yeast grew normally. It was found, however, that at a given ammonium concentration, increasing the pH increased the toxicity. Moreover, at any pH above 6.8, increasing the ammonium concentration enhanced the toxicity. Successful fermentation was not possible above a pH of about 7 when the ammonium concentration was greater than 0.1 normal. Since the ammonium ion was shown to be nontoxic in the concentrations studied, the data indicate that the toxicity arising from the use of ammonium salts of nontoxic anions is a function of the activity or chemical potential of molecular ammonia or ammonium hydroxide. The yield of glycerol increased as the pH approached 7, but above this value the toxicity of the ammonia inhibited the yeast fermentations. It was concluded that glycerol production by means of fermentations employing ammonium hydroxide as the alkalinizing agent would be unsatisfactory because of the poor glycerol yields obtained at the pH levels required for satisfactory fermentations.

The use of sodium carbonate or sodium hydroxide solutions as alkalinizing agents, in contrast with the use of ammonium hydroxide, was successful in producing satisfactory fermentations. It is desirable to keep to a minimum any necessary dilution of the fermenting medium. Sodium hydroxide solutions were preferable to sodium carbonate solutions for automatic pH-controlled operations because of the greater solubility and alkalinizing capacity of the sodium hydroxide. When the pH was controlled automatically at pH = 8.0 by a sodium hydroxide solution, a glycerol yield of 22.8 per cent of the sugar weight was found.

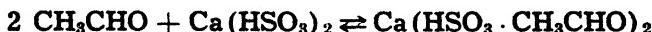
The production of glycerol by yeast fermentation in the presence of sulfite depends upon the fixation of acetaldehyde as a bisulfite addition product. However, it is necessary that the bisulfite concentration be kept at a low level since bisulfite is toxic to yeast.

It was found experimentally that the solubilities of calcium sulfite, magnesium sulfite, and of the bisulfite ion are functions of the pH. It was predicted that these salts should favor glycerol production in yeast fermentations, and that the toxicity, acetaldehyde fixation, and the subsequent glycerol yield should also be functions of pH. It was found that the bisulfite toxicity depended very markedly on the pH of the media, while the effect of pH on the glycerol was not so definite.

Both calcium sulfite and magnesium sulfite were found to be effective in fixing acetaldehyde in yeast fermentations for glycerol production. The fermentations were conducted in slightly acid media, and it was shown that increasing the initial sugar concentration decreased the percentage glycerol yields, the latter being an exponential function of the initial sugar concentration. The maximum extrapolated glycerol

yield was, in each case, about 26 per cent of the sugar weight as the initial sugar concentration approached zero. The time for the completion of the fermentations varied from about 1 to 4 days, depending upon the initial sugar concentration.

It was found that the concentration of the acetaldehyde-bisulfite complex approached a maximum in fermentation media utilizing calcium sulfite or magnesium sulfite for acetaldehyde fixation. The data obtained indicated that the extent of acetaldehyde fixation and hence the glycerol yield from fermentations involving calcium sulfite were controlled by the following equilibrium relation:



A similar equilibrium was indicated for magnesium sulfite. As the concentration of the complex increases, the concentration of acetaldehyde or its equivalent would also increase. The increased acetaldehyde concentration would allow its more extensive reduction to ethanol by means of glyceraldehyde phosphate during the biological dissimilation of the sugar. Such an operation would result in a decreased glycerol yield. It was concluded that the success of the glycerol fermentation in the presence of slightly soluble sulfites may depend on the decomposition or deactivation of the acetaldehyde-bisulfite complex during fermentation.

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# THE HOST RELATION OF THE COTTON FLEA HOPPER<sup>1</sup>

EPHRIAM HIXSON

From the Department of Zoology and Entomology, Iowa State College

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The cotton flea hopper, *Psallus seriatus* Reut., is one of the most important pests attacking cotton in the United States. It occurs in 22 states and the District of Columbia, extending from California to Florida and from Texas to Minnesota, and includes most of the cotton-growing states.

The first extensive injury to cotton by this insect occurred in 1926. This outbreak was probably brought about by the increased crop acreage in the United States between 1915 and 1926, which made possible the production of large areas of the weed hosts on the disturbed soils. On these weed hosts, large numbers of the cotton flea hopper were developed, and in turn migrated into cotton where they fed and caused injury.

The cotton plant is injured by the feeding of the adults and nymphs of the cotton flea hopper. They pierce the tissue of the growing tip of the cotton plant and suck the sap. This feeding causes the death and shedding of the small squares. The plant responds to the injury either by making a whiplike growth with scarcely any branches, or a rank vegetative growth. In both cases the plants are characterized by the absence of squares and blooms. The injury to cotton may be too small to measure or it may be a total loss of the crop.

The cotton flea hopper is known to feed upon 138 species of plants, which are distributed in 28 families. The most important species of host plants do not occur in all parts of the infested area, and likewise, the cotton flea hopper is not co-existent with its known hosts.

In Oklahoma, the cotton flea hopper occurs on 87 species of plants which are distributed in 24 families. The most important host plants are contained in four genera as follows: *Oenothera*, *Monarda*, *Solanum*, and *Croton*. Some species of plants are important in the western part of Oklahoma, while other species are important in the eastern part, and there is an overlap of species in the central portion encroaching eastward and westward, as the case may be.

Unfed flea hopper nymphs hatching from hibernated eggs were successfully reared to maturity on 25 species of plants, and lived from 2 to 16 days on 41 other species. The unfed nymphs, which were caged the same way as those on the plants, but denied access to plants, died within 24 hours.

Nymphs of the cotton flea hopper hatching from hibernated eggs in *Croton capitatus* Michx. were found feeding on plants growing adjacent to that species. The most common plant upon which they were found was

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*Ranunculus parviflorus* L.; however, *Oenothera laciniata* L. was commonly fed upon but was not present in as great numbers as *Ranunculus parviflorus*. Where these two species of plants were found growing 100 feet from Croton, no cotton flea hoppers were present. Migration of the nymphs of cotton flea hoppers does not seem to be characteristic, and they feed upon the plants which are present near the hibernation host.

The adults of the cotton flea hopper are, unlike the nymphs, distinctly migratory. In fact, preferred host plants which grow near the hibernation host and are not infested by the nymphs hatching from the hibernation host, are infested by adults developing from the nymphal population. There is a decided preference of hosts shown by the adult.

The most important host plants of the cotton flea hopper are divided seasonally as follows:

1. The Early Spring plants upon which the nymphs hatching from the hibernated eggs develop. The most important of these in Oklahoma are *Oenothera laciniata* and *Ranunculus parviflorus*, the former throughout the state and the latter in the eastern part. There are other local species of importance, and perhaps many that are not known.

2. The Late Spring and Early Summer host plants. These plants serve as host to the first generation of adults which have developed upon the Early Spring hosts. The more important species of this group are the various Monardas, *Solanum elaeagnifolium* Cav., in some cases *Oenothera laciniata* L. which may be in a desirable condition for feeding and development. The most important species of this group are *Monarda fistulosa* L. and *Monarda dispersa* Small on the upland, *Anthemis cotula* L. in the wooded, damp, and marshy areas of the river flood plains. In western Oklahoma, *Croton texensis* (Kl.) Muell. Arg. and *Solanum elaeagnifolium* are very important in this group and continue throughout the season.

3. The Late Summer hosts. This group contains those plants which act as host from July until frost and some of them serve as host for the hibernating eggs. The most important of these are the various species of Croton. In the eastern part of Oklahoma, *Croton capitatus* Michx. is the most important species. The infestation of this plant continues from about June 10 until frost, which corresponds with the blooming period. In the western area of Oklahoma, in the sandy regions, *Croton texensis* is the most important. Its infestation continues from the latter part of May until frost. *Croton Lindheimeranus* Scheele occurs most commonly in the central prairie regions of Oklahoma and is found growing with the two former species of Croton, but it is most commonly found in small grain stubble in the northern part of the cotton belt. The other Crotons are of minor importance in Oklahoma, due to their restricted range. *Solanum elaeagnifolium* is important in the western part of Oklahoma, but is usually not heavily infested. *Monarda punctata* L. is important in sandy areas during July, but is in restricted areas and therefore not of more than local importance.

The Crotons are the most important hosts in which the cotton flea hopper eggs hibernate. There are other plants known to successfully harbor the eggs during the winter, but in all cases the number of nymphs hatching from those plants is small. These plants include cotton (*Gossypium hirsutum* L.); *Helianthus* sp., *Ambrosia* sp., and *Solanum elaeagnifolium*. In the western areas *Croton texensis* is the most important winter host; in the eastern area of Oklahoma and Texas, *Croton capitatus* is the most important, and the latter species seems to be rather important eastward in the United States, while in South Carolina *Croton glandulosus* L. is considered as the most important hibernation host.

Cotton, *Gossypium hirsutum*, is attacked by adult cotton flea hoppers, moving from the Late Spring and Early Summer hosts which have matured, in search of the Summer and Fall hosts. If the preferred hosts are not present in proper condition for feeding and breeding, they remain in cotton for a greater or less time until such hosts are available. The time spent in cotton apparently depends upon the rate of growth, the size of the field, and the nearness of the preferred Crotons.

The population of cotton flea hoppers in cotton is never as large as the population in the weed hosts. The maximum number of both adult and nymphal cotton flea hoppers found in cotton is about 200 per 100 plants, which is equivalent to a total of about 600 to 100 sweeps of the net. In *Monarda fistulosa*, *Monarda dispersa*, and *Croton capitatus*, the maximum number of adults per 100 sweeps of the net was 600, with about an equal number of nymphs, while in *Croton texensis* the number has been estimated at from 1,000 to 2,000, but by actual count on the basis of one sweep per sample, the number found was a minimum of 1,300; the maximum was 13,800, and the average was 6,780 adults per 100 sweeps of the net. A sweep into one plant of *Croton texensis* yielded 123 adult cotton flea hoppers. In most cases the samples contained about equal numbers of adults and nymphs.

The hosts of the cotton flea hopper grow almost exclusively on disturbed soil such as over-grazed pastures, abandoned fields, road sides, fence rows, neglected fields, terraces, and cut-over timber and river flood plains. The plants are unable to compete successfully with good stands of grass, and have not been found growing in well-cared-for meadows, or in properly managed pastures. In well-cultivated fields they are not a problem, but in wheat stubble following harvest, the Crotons appear in large numbers and dominate the area until plowed or until frost comes.

The control of the cotton flea hopper is one of land utilization in which pastures are not over-grazed, where terraces are either grassed or cultivated, and where fields are properly cultivated.

The Crotons are the key plants in the successful development of the cotton flea hopper. Their control should not be a difficult problem, due to their inability to compete with grasses and other dominant plants. Probably the easiest method of control would be to mow all infested areas and encourage grasses to grow.

MEAT IN NUTRITION. XVII. CONCENTRATION OF SUGAR IN  
BLOOD OF PREGNANT RATS FED A DIET CONTAINING  
DRIED AUTOCLAVED PORK MUSCLE.<sup>1</sup>

FLORENCE PEN HO

*From the Department of Foods and Nutrition, Iowa State College*

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Gestational failures have been consistently produced in albino rats by workers in the Nutrition Laboratory of the Foods and Nutrition Department at the Iowa State College by feeding semisynthetic diet known as Pork I. This ration contained 25 per cent dried autoclaved pork muscle as its main source of protein. The mortality of both mothers and young was very high as compared with that observed in control rats reared on the stock ration (Steenbock V). The characteristic syndrome noted in the reproductive disturbance in the rats fed the pork diet is similar to that of human eclampsia.

On autopsy, the livers of the animals receiving the Pork I diet showed definite changes. The liver increased in size, was yellow in color, and friable and spongy in consistency. Microscopic examination of sections of liver showed a fatty infiltration that became more pronounced when the disorder appeared among the gravid animals (Armstrong,<sup>2</sup> 1939). In this case, fatty degeneration developed, also. The chemical analysis of the organ revealed a high fat content. The infiltration of fat in the hepatic cells may disturb the processes of glycogenesis or glycogenolysis which in turn may cause fluctuations in the concentration of glucose in the blood. Therefore, it seemed that a study of the blood sugar values of the pregnant rats fed the pork ration should yield some information regarding carbohydrate metabolism.

The purpose of the present investigation was to determine the concentration of sugar in the blood of pregnant rats fed the Pork I ration and to compare it with that in rats receiving the control stock ration.

In the experiment, 85 female rats were used. They were divided into two experimental series.

The pregnant groups in the first experimental series consisted of a control group fed the Steenbock V ration. The other experimental group received the Pork I ration. In order to determine whether pregnancy itself exerted an effect upon blood glucose levels, pregnant and virgin females were studied in the control and experimental groups. The pregnant animals were killed on the 21.5 day of the second gestation. They were standardized by means of starvation for 13 hours; 4 gm. of their own diet was fed at the end of this period; and then the animals were stunned

<sup>1</sup>Original thesis submitted June, 1941. Doctoral thesis number 635.

<sup>2</sup>Armstrong, W. E. 1939. Meat in nutrition. XV. Certain characteristics of gestational performance in albino rats fed a diet containing dried autoclaved pork muscle. Doctoral Thesis No. 524, Iowa State College Library, (1939). [Unpublished.]

4 hours after the feeding. The blood was drawn immediately for chemical analysis. The virgin females were killed when they were as old on the average as the pregnant animals in that group. They were also standardized before autopsy.

Since the control and experimental diets contained different ingredients, their respective digestibility and absorbability might be different. In order to minimize possible variations from these sources, a second series of experiments was planned. In this experiment, the quantity and quality of food fed after starvation was kept constant by administering 2.5 cc. of 50 per cent glucose solution to each rat instead of giving it its own ration.

In the second series of the experiment, both pregnant starved and pregnant glucose-fed groups of rats were studied. Each group was further subdivided on the basis of the control and experimental diets used in rearing the rats.

Results in Series I showed that the average concentration of sugar in the blood of virgin rats fed the Pork I experimental diet was 144.1 mg. per cent in contrast to 123.2 mg. per cent in the blood of the virgin animals receiving the Steenbock V control ration. It seemed that the experimental rats had a higher concentration of blood sugar than the control rats, but analysis of variance showed that the difference was not significant.

To determine whether pregnancy *per se* affected the concentration of sugar in the blood, virgin and pregnant rats fed the same diet were compared under the same standard conditions. The blood sugar values in the control group averaged 123.2 and 92.7 mg. per cent, respectively; in the experimental group, 144.1 and 97.4 mg. per cent, respectively. Thus, the quantity of blood sugar appeared to be lower in gravid rats fed both diets than it was in virgin animals. The differences noted in the level of blood glucose in pregnant and nonpregnant rats proved significant upon analysis of variance. Undoubtedly, the metabolic needs of the feti were responsible for the low blood sugar values observed in the pregnant group.

When the average quantity of sugar in the blood of the pregnant rats fed the Pork I diet (97.4 mg. per cent) was compared with that in the blood of pregnant rats fed the Steenbock V diet (92.7 mg. per cent), no marked difference was noted, and analyses showed that the variance within the groups was greater than that between them.

In order to determine the basal levels of the sugar concentration in the blood, both the control and experimental groups were starved for 13 hours. The blood sugar values at the end of this period averaged 58.4 and 73.9 mg. per cent, respectively. Statistical analysis showed that the difference was not significant.

Of the rats that were fed the glucose solution, the blood sugar concentration in the pregnant control group was 56.2 mg. per cent, and in the rats reared on the pork diet, 75.7 mg. per cent. Analysis of the variance indicated the difference was significant. It seemed strange that a sig-

nificant difference should exist in this instance, when the difference occurring in the levels of sugar observed in the blood of the two pregnant groups studied in the first series was not significant.

In the first series, fetal development was comparable in the experimental and control groups. The number of living feti found in the uteri of the experimental group was 8.2; in the control group, 10.5. However, in the second series of glucose-fed rats, there were only 5.9 feti alive in the uteri of the pork rats in contrast to 11.5 in the Steenbock V rats. Before it could be said that the blood of the pork rats was hyperglycemic, it was necessary to determine whether or not differences in fetal metabolism were affecting the material blood values. By a re-sorting of the data that made it possible to examine the concentration of glucose in the bloods of paired sister mates reared on the two diets, it was found that the larger the number of feti present, the lower was the concentration of blood sugar. If the number of feti produced by each member of the pair happened to be the same, the glucose concentration was also approximately the same. Therefore, the difference originally observed, while real, was not one that pointed to a hyperglycemia in the pork rats.

From the data presented above, the following conclusions seem warranted:

1. Pregnancy *per se* lowers the concentration of sugar in the blood;
2. The greater the number of living feti present in the uterus, the lower is the concentration of sugar in the maternal blood;
3. The level of blood sugar remains normal in rats fed the pork-containing diet until the 21.5 day of gestation, even though the concentration of glycogen is known to be subnormal; and
4. Whether the concentration of sugar in the blood is disturbed when acute pregnancy disease develops remains to be demonstrated.

# SOIL-INHABITING FUNGI ATTACKING THE ROOTS OF MAIZE<sup>1</sup>

WEN-CHUN HO

From the Department of Botany, Iowa State College

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Several pathogens were known to attack the roots of maize, but the effect of these pathogens was not well understood. During the course of the present study various organisms were found to be associated with the decayed seeds or infected roots, mesocotyls, plumules, and crowns of maize. The organisms found most often on the decayed seeds were: *Pythium debaryanum* Hesse, *Gibberella saubinetii* (Mont.) Sacc., *Penicillium oxalicum* Currie and Thom, *Trichoderma lignorum* (Tode) Harz., and *Fusarium* spp. Those occurring on diseased roots were *Pythium debaryanum*, *Pythium graminicola* Subr., *Gibberella saubinetii*, *Helminthosporium sativum* P. K., and B., *Rhizoctonia solani* Kuhn, *Trichoderma lignorum*, *Fusarium moniliforme* Sheldon, and *Fusarium* spp. Isolations made from infected mesocotyls yielded chiefly *Gibberella saubinetii*, *Rhizoctonia solani*, *Helminthosporium sativum*, *Penicillium oxalicum*, *Trichoderma lignorum* and *Fusarium* spp., and those from diseased plumules yielded *Gibberella saubinetii*, *Fusarium* sp., and *Rhizopus* sp. The organisms that appeared on crowns were *Diplodia zeae* (Schw.) Lev., *Gibberella saubinetii*, *Penicillium oxalicum*, *Aspergillus niger* Van Tieghem, *Fusarium moniliforme*, *Trichoderma lignorum*, and *Fusarium* spp.

These organisms may be divided into three groups based on their pathogenicity on maize in the greenhouse and under field conditions: (1) Highly destructive, *Pythium debaryanum*, *Pythium graminicola*, and *Gibberella saubinetii*; (2) moderately destructive, *Rhizoctonia solani*, *Helminthosporium sativum*, *Diplodia zeae*, and *Penicillium oxalicum*; (3) slightly to none, *Aspergillus niger*, *Fusarium moniliforme*, *Trichoderma lignorum*, *Rhizopus*, sp. and *Fusarium* sp. Pathogenicity was more constant in *Pythium debaryanum* and *P. graminicola* than in *Gibberella saubinetii* and *Helminthosporium sativum*.

The pathogenicity of *Pythium debaryanum* and *Helminthosporium sativum* on maize was established for the first time. The former appeared in the early growing season and caused root-tip necrosis of fine rootlets, while the latter occurred on the infected roots and mesocotyls in the mid-growing season.

Under field conditions the various soil-inhabiting pathogens usually attacked the plants in combination. Under such conditions a combination of symptoms frequently occurred, but in other cases symptoms of one organism might predominate over the other. Results obtained from the greenhouse studies on the combinations of symptoms indicated that when two active pathogens occurred together, the symptoms of each organism

<sup>1</sup> Original thesis submitted June, 1941. Doctoral thesis number 638.

were distinct and the severity of the disease rating generally increased over the corresponding rating of single cultures of any one of them. In this case, the destructive effect was distinctly additive. In combinations of an active pathogen with a weak pathogen or a saprophyte, the severity of the disease was found to be less than that of the active pathogen alone. The destructive effect in this combination was subtractive. In some cases the symptoms of one organism were predominant because the conditions that prevailed were unfavorable to the other organism. The pathogenicity of almost all active pathogens was somewhat suppressed when any one of them occurred simultaneously with *Trichoderma lignorum*. The destructive effect in this combination was inhibitive.

Under field conditions more than one pathogen usually appeared on the underground parts of the plants simultaneously and seemingly in succession as the season advanced. Therefore, an attempt was made to determine the active pathogens that caused the initial infection, the secondary invaders that followed the initial infection, and the saprophytes that grew on the infected tissue. The relation between the occurrence of the specific pathogens and their symptoms was investigated. In most cases *Pythium debaryanum* became parasitic before and following seedling emergence, causing seed decay, stunted growth, and a tip necrosis on the primary and some of the seminal roots. When the soil reached a higher temperature, *P. debaryanum* caused only slight injury, while *P. graminicola* became aggressive and appeared on the tips of most of the roots, inducing a rapid necrosis and a brown, water-soaked appearance. At the same time, *Gibberella saubinetii* and *Rhizoctonia solani* often occurred on the lower part of the mesocotyl, and later caused a discoloration and cortical necrosis of the roots. As the season advanced, other organisms, such as *Helminthosporium sativum* and *Penicillium oxalicum*, produced further necrotic lesions on the mesocotyl and basal part of the roots. Still later, *Fusarium moniliforme*, *Aspergillus niger*, *Trichoderma lignorum*, and *Fusarium* spp. were observed in the root lesions. The lesions on the roots and mesocotyl were followed by a number of saprophytic soil-inhabiting organisms, chiefly species of *Fusarium*. When the maize matured, *Diplodia zeae*, *Penicillium oxalicum*, *Aspergillus niger*, *Fusarium* spp., etc., might develop extensively on the crown, leaving no evidence of the organisms that prevailed earlier.

The response of fourteen open-pollinated varieties, fifteen inbreds, fifteen single crosses and twenty-three double crosses of maize studied in the greenhouse and field showed a considerable variation in their relative resistance to *Pythium graminicola* and *P. debaryanum*. Among varieties studied, Kossuth County Reliance and Stern Yellow Dent were susceptible to *P. graminicola* and *P. debaryanum*, while Black Yellow Dent and Krug were more resistant. The majority of the fifteen inbreds studied were susceptible to both species of *Pythium*. Inbreds Ldg (k), Black 349, Lancaster 289, Osterland 426, and Hy showed some resistance. Single crosses that were combinations of less susceptible inbreds, such as Ldg (k) x Pr. (Osf), Lancaster 317 x Black 349, Osterland 420 x Osterland

426, and R4 x Hy, were resistant to both species of *Pythium*, while single crosses that were combinations of very susceptible inbreds, such as Iodent 205 x Black 345 and Iodent 205 x Iodent 234, were susceptible. Among twenty-three hybrids, Hi-bred 307, Hi-bred 322, etc., were more susceptible to *P. graminicola* and *P. debaryanum*, while Iowa hybrids, particularly Iowa 13, Iowa 931, and Iowa 939, were more resistant. National hybrid 110 (Edge) was susceptible to *P. graminicola* but more resistant to *P. debaryanum*.

Varieties, inbreds, single crosses, or double crosses that were very susceptible to *P. graminicola* also tended to be very susceptible to *P. debaryanum*, and those that were resistant to *P. graminicola* also tended to be resistant to *P. debaryanum*. A comparison of the relative resistance of open-pollinated varieties, inbreds, single crosses, and double crosses to *P. graminicola* and *P. debaryanum* revealed that, in general, open-pollinated varieties and double crosses were least susceptible, while single crosses were less susceptible than inbreds. Some of the open-pollinated varieties possessed more resistance than the hybrids.

In general, the infection became more severe in plants held for 10 days at 8° C. and then transferred to 22°-27° C., than on plants held continuously at the higher temperature. The infection on varieties Triple Dent, Silver Dent, Silver King, and Gold Mine, however, was more severe at continuous high temperatures. At high temperatures the injuries caused by *P. debaryanum* were distinctly diminished.

The disease rating of susceptible open-pollinated varieties, such as Kossuth County Reliance and Stern Yellow Dent, was reduced from 22 to 69 per cent; of susceptible inbreds, such as Iodent 205 and Iodent 234, from 34 to 83 per cent; and that of susceptible double crosses, such as Hi-bred 307 and Hi-bred 322, from 26 to 60 per cent by seed treatment. The effect of seed treatment was more pronounced at low temperatures than at high and was more conspicuous upon the susceptible ones than upon the resistant ones. At high temperatures the infection caused by *P. debaryanum* was commonly retarded by seed treatment, while the root necrosis caused by *P. graminicola* was not appreciably affected.

# DISTRIBUTION OF SALT IN BUTTER AND ITS EFFECT ON BACTERIAL ACTION<sup>1</sup>

WESLEY HENRY HOECKER

*From the Department of Dairy Industry, Iowa State College*

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The retarding effect of salt on the activity of many micro-organisms is generally recognized, and various investigators have presented evidence showing that the addition of salt to butter improves the keeping qualities. However, salted butter sometimes deteriorates through bacterial action.

Since the average concentration of salt in the serum of butter often is sufficient to inhibit growth of most bacteria producing spoilage in the product, the ability of micro-organisms to grow and produce defects in salted butter suggests that possibly not all of the serum in a churning contains the same concentration of salt. This would permit growth of bacteria in micro portions of butter containing little or no salt, while in other portions, containing higher concentrations, growth would be inhibited. Because of the spoilage of salted butter through bacterial action, the distribution of salt in butter and its effect on bacterial action were investigated, using a micro technique for the salt determinations. The investigations involved the development of a micro method for the determination of salt in butter, studies on the salt distribution in butter, and studies on the effect of salt distribution on bacterial action.

In general the micro method is carried out as follows: An approximately 0.2-mg. ( $\pm 0.05$  mg.) portion of butter is picked under a low power microscope and weighed on a microbalance. The butter is then ashed, and the ash and salt are taken up in a water-alcohol solution and transferred to a spot plate. A known amount of standard sodium chloride is added, and the mixture is titrated with a 0.01 N silver nitrate solution under a fluorescent type lamp, using dichlorofluorescein as the indicator.

In studying the distribution of salt in butter both commercial and experimental churnings were used. With each churning ten micro samples from a 15-g. portion were analyzed. In some samples of normal commercial butter the salt was very uniformly distributed, while in other samples the salt was not uniformly distributed. With most samples a correlation was noted between the salt distribution and incorporation of the moisture. With normal commercial churnings no significant differences in salt distribution were noted between the butter before and after printing with equipment which tended to rework the butter. Studies on the salt distribution in butter removed from commercial churnings at different times during the working process showed that, as the working process continued, the distribution of salt became more uniform. In some samples of putrid commercial butter the salt was very unevenly dis-

<sup>1</sup>Original thesis submitted June, 1941. Doctoral thesis number 632.

tributed, while in other samples the salt was very uniformly distributed. In mottled commercial butter the light-colored portions usually contained less salt than the dark-colored portions.

In studying the distribution of salt and moisture in butter, both the salt and the moisture were determined on the same micro samples. With each churning, analyses were made on ten samples, each of approximately 0.3 to 0.5 mg., from a 15-g. portion of butter. The moisture contents were determined by picking and weighing the samples in the usual manner, drying them in a 100°C. oven, and allowing the dried samples to cool under room conditions for 30 minutes before weighing; the losses in weight during drying represented the moisture contents. In some samples large water droplets appeared on the freshly cut surfaces of the cold butter. These droplets were analyzed for salt by removing a portion of each droplet with a very small weighed capillary tube, sealing both ends of the tube and then reweighing. The material was removed from the capillary tube by crushing it with a small stirring rod in a spot plate depression containing a few drops of water.

Much larger variations occurred in the moisture contents than in the salt contents of the micro samples of butter. Also, much larger variations occurred in the salt contents of the serums in the micro samples than in those of the large moisture droplets. The salt contents of the serums calculated from the micro analyses were slightly lower than the values calculated from the macro analyses. The salt contents of the large moisture droplets usually were higher than those of the serums, calculated either from the micro or the macro analyses.

The effect of salt distribution on bacterial action in butter was studied with small laboratory churnings. Pasteurized cream was inoculated immediately before churning with lactic acid-producing organisms, or the pasteurized cream, the wash water, or the butter was inoculated with organisms producing a flavor defect in butter. The cream was churned in a small hand churn. Each churning was used to prepare samples of salted butter with the salt well distributed and poorly distributed and samples of unsalted butter that were thoroughly worked and poorly worked. The butter was held at 15.5°C. The activity of the micro-organisms was determined by the change in numbers of organisms as shown by plate counts or by the Burri smear culture technique, by the microscopic examination of butter serum, by the change in pH of the butter serum, and by the development of flavor defects in the butter.

Salt tended to inhibit bacterial action in butter, the greatest inhibition occurring in butter in which the salt was well distributed. The micro-organisms were most active in poorly worked unsalted butter.

The distribution of salt in butter on a micro basis should be considered a factor that definitely influences the action of micro-organisms in butter; the more thoroughly the salt is distributed the greater is the inhibitory effect of the salt.

RELATIONSHIP OF THE LIPOLYTIC AND PROTEOLYTIC  
ACTIVITIES OF VARIOUS PENICILLIA TO THE  
RIPENING OF BLUE CHEESE<sup>1</sup>

CHRISTIAN JENSEN

*From the Department of Dairy Industry, Iowa State College*

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Studies on the penicillia employed in blue cheese ripening were made from the standpoint of the relationship of their lipolytic and proteolytic activities to the time of ripening, to the quality of ripened cheese, and to determine the possibility of employing cultural methods in selecting suitable strains of penicillia for ripening.

The work reported involves (a) a comparison of methods employed in determining lipolytic and proteolytic activities of various penicillia, (b) estimation of lipolytic and proteolytic activities of various penicillia, and (c) inoculation of cheese curd with the molds to determine the effect of lipolytic and proteolytic activities of various penicillia on the ripening of blue cheese.

RESULTS OBTAINED

The lipolysis of butterfat and cottonseed oil by various penicillia was more readily detected with the nile blue sulfate technique than with the natural fat technique. Although the natural fat technique resulted in greater colony growth, the nile blue sulfate technique usually gave the more distinct differentiation between unhydrolyzed and the hydrolyzed fat.

The modified nile blue sulfate technique for determining the lipolytic activities of various penicillia on butterfat and cottonseed oil gave a more distinct differentiation between the unhydrolyzed and the hydrolyzed fat than the natural fat technique.

No appreciable difference in effect on the detection of lipolysis by various penicillia was noted when 0.5 ml. or 1.0 ml. of a 3 per cent fat emulsion was used per plate (15 ml. agar). However, when 1.5 ml. fat emulsion was used the uniformity of lipolysis was affected.

With the nile blue sulfate technique for determining lipolysis of butterfat by the penicillia, the best results were obtained when the media contained 1 part of the dye in 15,000 or 20,000 parts of agar. Higher concentrations of the dye caused marked inhibition of mold growth, while lower concentrations failed to color the fat properly.

Proteolysis was more readily detected on Czapek's solution milk agar when the cultures were incubated: (a) at 28° C. rather than at 20° or 12° C.; (b) at 21° C. in the air 4 days, followed by 4 days in an atmos-

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<sup>1</sup>Original thesis submitted June 3, 1940. Doctoral thesis number 568.

sphere charged with carbon dioxide rather than at 21° C. in air for 6 days or 8 days.

At 21° C. proteolysis was more readily detected on Czapek's solution milk agar than on beef infusion milk agar when the cultures were incubated 4 days in the air, followed by 4 days in carbon dioxide, or when they were grown for 8 days in carbon dioxide.

Somewhat more clear-cut results were obtained with the carbon dioxide technique (incubation on Czapek's solution milk agar with incubation at 21° C. for 4 days, followed by 4 days in carbon dioxide) in the detection of proteolysis than with the acidified milk agar technique.

There was considerable variation in the lipolytic activities of various penicillia on butterfat and cottonseed oil, as determined by the natural fat and the nile blue sulfate techniques; the intensity and uniformity of lipolysis of the cultures ranged from nonlipolytic to very pronounced lipolytic.

There was considerable variation in the lipolytic activities of various penicillia on different triglycerides according to the nile blue sulfate technique. Only a few cultures hydrolyzed tripalmitin, while all readily hydrolyzed tributyrin and trivalerin. As the molecular weights of the triglycerides increased, variations in lipolytic activities of the cultures became more conspicuous. Some cultures showed gradual declines in their lipolytic activities, whereas others declined sharply on the triglycerides beginning with tricaprin. Most cultures were actively lipolytic on the triglycerides from tributyrin up to and including tricaprin but several showed no lipolysis with trilaurin.

There was considerable variation in the toxic effect of different triglycerides on various penicillia. In general, the triglycerides that exhibited the most pronounced toxicity, in declining order of their effect, were tripalmitin, tributyrin, trivalerin, tricaprin, trilaurin, trimyristin, and tripalmitin. The least toxic were triheptylin, tricaproin, tricaprylin, and triolein.

The lipolytic activities of various penicillia on butterfat were retarded when the cultures were grown in an atmosphere consisting of 3 parts air and 1 part carbon dioxide, while their activities were markedly accelerated in 3 parts of air and 1 part nitrogen and slightly accelerated in 3 parts carbon dioxide and 1 part nitrogen.

The lipolytic activities of various penicillia on butterfat declined slightly when 4 per cent sodium chloride was added to the medium.

Certain penicillia showed greater lipolytic action on cottonseed oil at 25° C. than at 9° to 10° C.; more rapid mold growth also occurred at the higher temperature.

There was considerable variation in the proteolytic activities of various penicillia as determined by the acidified milk agar and the carbon dioxide techniques. There was a general agreement between the results obtained with the two techniques.

The rates of growth and of proteolysis of certain penicillia were

affected by different growth conditions. The cultures grew more slowly but showed greater proteolytic activities in air at 28° C. than at 19° or 12° C.; the cultures were somewhat retarded in growth, but proteolysis was unaffected when grown at 28° C. in an atmosphere in which 10 per cent of the air had been replaced by carbon dioxide; culture growth and proteolysis at 28° C. were almost stopped in an atmosphere saturated with carbon dioxide; growth usually was unaffected but proteolysis was accelerated at 28° C. in an atmosphere consisting for the most part of nitrogen.

The inoculation of cheese curd with dry bread mold cultures of penicillia was the only satisfactory method of establishing the penicillia in cheese. Suspensions of spores from agar slopes or plate cultures failed to introduce sufficient mold spores for establishment of the inoculated cultures.

Blue cheese ripened with pronounced lipolytic and proteolytic penicillia usually showed better mold growth and earlier development of a good flavor than those ripened by less active molds. The bitter and green flavors often noted, during the early period of ripening, in cheese inoculated with the pronounced lipolytic and proteolytic penicillia gradually disappeared, and good flavors usually resulted. When the cheese were ripened with slightly lipolytic and proteolytic penicillia, they frequently showed poor mold growth and flavor development. However, the results were not always consistent, since in a few instances certain pronounced lipolytic and proteolytic penicillia resulted in cheese with poor flavor.

There were marked variations in the flavors of cheese ripened with different strains of penicillia. Cheese ripened with *P. roqueforti* usually showed good mold growth but not always good flavor. Cheese ripened with a culture of *P. chrysogenum* showed poor growth and flavor; cheese ripened with *P. gorgonzola* showed from good to poor growth and usually from fair to poor flavor; one culture of *P. stilton* usually produced good growth and from good to fair flavor in the cheese; while one culture of an unidentified *Penicillium* gave fair growth and flavor.

# A MICROMORPHOLOGICAL APPROACH TO THE STUDY OF SOILS<sup>1</sup>

JOSEPH REX JOHNSTON

*From the Department of Agronomy, Iowa State College*

Received June 10, 1941

Soil structure classification and nomenclature of morphological units in soils have received considerable attention by soil scientists in recent years. Dr. W. Kubiena has introduced the term "elementary soil fabric," which is essentially the description of a relation existing between the soil skeletal material and soil colloids. The six most important elementary soil fabric types as described by Dr. Kubiena are discussed. Some of these fabric types occur in some soils of the United States and are described and illustrated. The possibilities of using Dr. Kubiena's concept of soil fabrics in soil structure classification and nomenclature are pointed out. It was concluded that knowledge concerning soil structure formation and genesis is far from adequate. Furthermore, it was pointed out that before an orderly system of soil structure classification can be developed, attention must be given to three phases of soil structure: (1) microstructure, (2) macrostructure, and (3) tertiary soil structure.

Thin sections of soils in their natural structural arrangement, as described by Ross, by Kubiena, and by Volk and Harper were used in some mineralogical and weathering observations on the Webster clay profile. The thin section studies were supplemented with some specific gravity mineral separations of the fine sand fraction from each of the principal horizons of the soil studied. It was found that specific gravity separations of the fine sand fractions provided a means of more detailed mineral study than thin sections alone. The thin sections can be used in making a good, rapid qualitative determination of the minerals in the soil and can be quickly made, whereas specific gravity determinations require considerable time. Furthermore, thin sections reveal the minerals in their natural positions in relation to the other minerals and constituents of the soil, information which is not apparent when the sample is mixed as for specific gravity determinations.

Thin-section studies of the principal horizons in nine soil types, representing seven great soil groups, were made. The great soil groups studied were the Gray-brown Podzolic, Red Podzolic, Yellow Podzolic, Prairie, Chernozem, Weisenboden, and Rendzina. The characteristics of soils as observed in thin section with the microscope were studied in relation to certain physical and chemical properties of soils such as mechanical composition, hygroscopicity, base exchange capacity and percentage organic matter. It was found that from thin-section observations, a general idea of the relative proportions of sand, silt, and clay in the soil

<sup>1</sup>Original thesis submitted June, 1941. Doctoral thesis number 627.

can be obtained. The amount and stage of decomposition of the soil organic matter can be roughly estimated from thin-section observations. The more obvious and outstanding features of soils that can be studied by thin sections are (1) movement of soil colloidal matter and other mobile constituents, (2) soil microstructure and porosity, and (3) the mineral composition in respect to whether there is an abundance or scarcity of the minerals high in calcium, magnesium, potassium, phosphorus, and iron. The thin section enables a worker to determine whether the soil colloid is composed largely of organic material, of the oxides of iron and aluminum, or of one of the clay minerals.

The use of the thin section as a technique in the study of soil classification has promise because thin sections can be made quickly and with a small outlay of equipment, and because they make possible a more accurate and complete description of soils. Furthermore, the thin section is small, compact, and easy to handle, all of which makes it possible to catalogue and file the sections for quick reference and study. In other words, a set of thin sections representing the principal horizons of the soil types mapped within a state or area would be an asset to an organization to whom knowledge of soils is valuable. Also, the thin section is of such a nature that it can be sent from place to place with a minimum of cost and care, thus facilitating comparative study, correlation, and classification of soils.

Nine plates illustrating the thin sections by photomicrographs and the mechanical composition, organic matter content, base exchange capacity, and complete pF curves of the principal horizons of the nine soil types are included. The thin sections and physical and chemical properties of each of the soil types studied are discussed.

# THE EFFECT OF INSECT CONTROL ON THE YIELD AND QUALITY OF COTTON PREMATURELY KILLED BY COTTON ROOT ROT.<sup>1,2</sup>

S. E. JONES<sup>3</sup>

From the Department of Zoology and Entomology, Iowa State College

Received May 22, 1941

Insect pests and cotton root rot are major problems of cotton production in Texas. Control measures are known for use in combatting the cotton flea hopper (*Psallus seriatus* (Reuter)) and the boll weevil (*Anthonomus grandis* Boh.), but more practical methods are needed for preventing losses caused by root rot (*Phymatotrichum omnivorum* (Shear) Duggar).

## EXPERIMENTAL

To the writer's knowledge this was the first study ever made to determine the effect of insect control on the yield and quality of cotton from plants killed by root rot before the first normal picking. It was an attempt to discover an indirect method of reducing root rot losses by enabling the plants to set a larger crop before death occurred. Since the flea hopper frequently destroys the first fruiting forms and thereby delays the crop, the insect infestation was controlled to see if the standard control measures would be profitable to use in fields where varying amounts of root rot occur.

The experiments were conducted at Cameron, Texas, during 1937 and 1938, and at Port Lavaca, Texas, during 1939. Plots were selected in fields where root rot was known to occur and there was already present an incipient flea hopper infestation. The treatments consisted of controlling the flea hopper with sulfur dust and the boll weevil with calcium arsenate dust on one plot and leaving another as an untreated check. Two 1-acre plots were used in each of four fields during 1937. The treatments were arranged in randomized blocks and replicated three times in three fields during 1938 and four times in four fields during 1939. These plots were approximately one-sixth acre in size. The yield data were subjected to analysis of variance. Insect infestation records were made throughout the season, and dusts were applied when needed. The control obtained was determined by the reduction in infestation and yield records. Plants which died during each week were marked with a dated tag. The cotton was picked from the dead plants at the time of the first normal picking, and from the plants which were living at that time in the 1938 and 1939 tests. The total yield from each group of plants was accurately weighed. All bolls which opened, regardless of size, were picked.

<sup>1</sup>Original thesis submitted June 4, 1940. Doctoral thesis number 559.

<sup>2</sup>Technical contribution number 654 of the Texas Agr. Exp. Sta.

<sup>3</sup>Division of Entomology, Texas Agr. Exp. Sta.

The quality of the lint and seeds was determined by specialists of the Texas A. and M. College from samples secured during 1938. The lint was ginned by Mr. D. T. Killough, classed by Mr. J. G. Powers, and tested for breaking strength and maturity by Miss Mary Anna Grimes. The seeds were analyzed by Dr. C. H. Rogers.

The rainfall in the sections where these investigations were conducted was below normal: 5.66 inches in 1937, 7.55 inches in 1938, and 14.86 inches in 1939. Cotton plants under these droughty conditions were small, shed many fruit forms, and matured early. Insect and root rot damage was probably less than average.

The flea hopper infestation was low in all fields during 1937 and 1938. The infestation was sufficient to warrant dust applications for a period of about six weeks during 1939, but the adequate control obtained, based on infestation records, did not result in significantly increased yields on plants prematurely killed by root rot in any of the four fields. There was a significant increase in yield on the plants which were living at the time of the first picking in one field. Boll weevils did practically no damage during 1937 or 1939. There was an incipient infestation during 1938, but the control obtained did not result in increased yields on the dead or living plants. Excessive shedding, due largely to a deficiency of soil moisture, partly accounts for the failure to make increased yields in spite of insect control. It appears that the yields secured from plants killed by root rot were as high as could be expected under similar conditions when insect damage is prevented.

Root rot began killing plants each year about the time the first blooms appeared and continued throughout the growing season. Records were kept on 59,337 plants which died during the nine weeks preceding the first normal picking. Nineteen per cent of these plants died during the last two weeks. The percentage of plants killed in the 1938 and 1939 experiments varied from 5.11 to 53.13 with an average of 29.39 in all fields.

The yield from 49,164 dead plants in the 1938 and 1939 tests was 229 pounds of seed cotton. Of that amount, 161 pounds or 70.3 per cent were picked from 10,496 plants which died during the last two weeks. The remaining 38,668 plants which died earlier in the season produced 68 pounds or 1.4 per cent of the total yield. The 118,126 living plants produced 4,638 pounds of seed cotton. Even though 29.39 per cent of the plants were killed by root rot, they produced 4.7 per cent of the total yield. In one field where 53.13 per cent of the plants died, they produced only 9.5 per cent of the total yield.

It required an average of 157 bolls from the dead plants to yield 1 pound of seed cotton as compared with 72 from living plants. The number necessary to weigh 1 pound varied from 105 on plants which died during the last week to 270 from plants which died five weeks before the first picking.

Quality determinations were made of seed and lint from plants which died during each of six weeks before the first picking and from plants

which were living at that time on both dusted and undusted plats in the 1938 experiments. The percentage lint in the seed cotton varied from 39.3 in samples from plants which died during the last week to 32.1 from plants which died six weeks before the first picking. The seed cotton from living plants averaged 40.1 per cent lint. The grade was slightly better from living plants than from those dying during the last two weeks. Samples from plants dying earlier in the season had a very low grade. The average staple length from living plants was slightly shorter than from plants which died during the last week, but was slightly longer than from plants which died during the second week before harvest. The average staple length from plants dying more than two weeks before the first picking was considerably shorter than from living plants. The body and uniformity of the samples from plants dying during the last week were about the same as from living plants, but were lower from plants dying earlier. The maturity index and percentage maturity were lower from all dead plants than from living plants, and decreased in relation to the length of time the plants died prematurely. The breaking strength, as determined by the Chandler bundle and flat bundle methods, was higher from plants which died during the third and fourth weeks than any other dead or living plants. The strength of individual fibers was not determined. The ammonia and protein percentages of seeds were slightly higher from plants which died during the last week than from living plants, but plants which died during the second week had the same percentage as living plants. The ammonia and protein percentages decreased considerably in plants dying more than two weeks prematurely. The percentage oil in the seeds was highest in samples from living plants and decreased in relation to the length of the time the plants died prematurely. There was no difference in the quality of the seeds or lint in samples secured from dusted and undusted plants.

Although the quality of the lint and seeds was low in samples from dead plants, this cotton made up only 4.7 per cent of the total yield secured from all plants. The very low quality cotton from plants dying more than two weeks before the first picking made up 1.4 per cent of the total yield. Since all bolls which opened were picked, it is probable that the quality of the lint and seeds in these samples was lower than if the regular farm practice of leaving the small bolls had been followed.

#### CONCLUSIONS

Low yields from plants prematurely killed by root rot were not materially increased by controlling the flea hopper or boll weevil under the insect conditions existing during 1937-1939, inclusive. Those plants produced only a small amount of low quality cotton when there was no insect damage. The factor which determines the profit to be made from controlling insects on land where cotton dies from root rot is the yield from living plants regardless of the percentage dying early in the season. If the yield from living plants is sufficient for profitable cotton production, and insects are damaging the crop, control measures should be used.

# EFFECT OF THE PRESENCE OF SALTS ON THE HYDROGEN OVERVOLTAGE OF SOLUTIONS<sup>1</sup>

JOHN NELSON JUDY

*From the Department of Chemistry, Iowa State College*

Received May 22, 1941

The direct method for measuring overvoltage has been used to determine the effect of the presence of salts in solution on the hydrogen overvoltage at a nickel electrode. The effect of adding various salts to water, 0.1 N sulfuric acid, and 0.1 N sodium hydroxide has been studied at different current densities.

In each case the measurements have been made by determining the overvoltage of the original electrolyte at a definite current density, adding a measured amount of a standard salt solution from a burette, and again determining the overvoltage. By the successive addition of small portions of the salt solution, the overvoltage over a wide range of concentrations for the salt was determined. The electrolyte was then replaced with more of the original solution, the current set at a new amperage, and the procedure repeated. During the addition of the salt solution the current was maintained constant. In each case the solutions added were either molar or half molar with respect to the salt. In order that the concentration of the original electrolyte should not change due to the diluting effect of adding the salt solution, the solutions added were made 0.1 N with respect to acid or base. The solutions were prepared by placing the calculated amount of the Reagent Grade of the salt in a 1-liter volumetric flask, adding 100 milliliters of 1 N sulfuric acid or sodium hydroxide, and diluting with water to the mark.

The effect of increasing the concentration of sulfuric acid, sodium hydroxide, or salts in pure water was studied. In every case the overvoltage was high when the concentration was low. As the amount of electrolyte was increased there was a drop in the overvoltage. This drop was very rapid at low concentrations and became more gradual as the solution became more concentrated.

Three types of salt solutions were added to 0.1 N sulfuric acid, namely, (a) a neutral salt, (b) basic materials, and (c) oxidizing agents.

(a) When neutral sodium sulfate was added to sulfuric acid, the overvoltage was gradually lowered as the concentration of the sulfate was increased.

(b) The addition of sodium hydroxide or salts of strong bases and weak acids to sulfuric acid in every case produced results that were very similar. As the amount of the added material was increased, there was a sharp increase in the overvoltage to a maximum followed by a de-

<sup>1</sup> Original thesis submitted July 16, 1940. Doctoral thesis number 584.

crease as the concentration was further increased. The maximum in most cases came close to the point at which equivalent amounts of acid and salt were present in the solution. In addition to sodium hydroxide, the other basic materials studied were sodium phosphate, potassium oxalate, sodium formate, sodium acetate, and sodium fluoride.

(c) The addition of oxidizing agents, sodium chlorate, sodium nitrate, and sodium dichromate to sulfuric acid solution each caused a lowering of the overvoltage. With the first two of these salts there was a sharp drop as the first of the oxidizing agent was added followed by a more gradual fall as the concentration was further increased. With sodium dichromate the lowering was very great with a small concentration of the salt when the current density was low. At higher current densities there was a lowering of the overvoltage but the concentration had to reach a higher value before the effect was noticeable.

The effect on hydrogen overvoltage produced by the addition of (a) neutral salts and salts of weak acids and (b) sodium dichromate to 0.1 N sodium hydroxide solution was studied.

(a) In every case of the addition of a neutral salt or the salt of a strong base and a weak acid to sodium hydroxide there was a gradual decrease in the overvoltage as the concentration of the electrolyte was increased.

(b) When the concentration of sodium dichromate in sodium hydroxide was increased, there was at first a slight increase in the overvoltage followed by a lowering at higher concentrations.

From the results obtained it can be seen that there is a direct relationship between the concentration of the solution and overvoltage. This is true in the case of sulfuric acid, sodium hydroxide, and salt solutions.

When a basic material was added to sulfuric acid solution, the overvoltage was increased to a maximum and then decreased as the concentration was further increased. There are, in all probability, two factors at work producing this effect, namely, (a) reduction of the hydrogen-ion concentration due to neutralization, and (b) an increase of the total concentration of the solution. The first of these should cause an increase in the overvoltage, and the second should cause it to decrease. At first the effect of neutralization of the acid predominates, and the overvoltage is increased. This rise continues until the effect of increasing the total concentration has the greater effect and causes the overvoltage readings to drop. When an acid salt, sodium dichromate, was added to 0.1 N sodium hydroxide, similar results were obtained. The same two factors combine to produce this effect. The increase at first is due to the neutralization of sodium hydroxide followed by a subsequent decrease at higher concentrations.

Since the addition of sodium sulfate, a neutral salt, to 0.1 N sulfuric acid has little or no neutralizing effect, the only factor at work in this case is the increase in the concentration of the solution. This accounts for the gradual lowering of the overvoltage that was observed. Similar results

were obtained when neutral salts or salts of weak acids were added to sodium hydroxide solution. The addition of the salt of a weak acid has little effect on the pH of a 0.1 N sodium hydroxide solution; thus the concentration of the solution is the most important factor.

The addition of oxidizing agents to sulfuric acid solution caused the overvoltage to be lowered. This would indicate that oxidation of hydrogen at the electrode surface was taking place with the result that polarization was decreased. Very little hydrogen is liberated at an electrode when the current density is low and a low concentration of an oxidizing agent such as the dichromate ion, is sufficient to produce a large depolarizing effect. Hydrogen is liberated more rapidly at higher currents, and a greater concentration of the oxidizing agent is necessary to produce any noticeable lowering of the overvoltage.

#### SUMMARY

1. In this investigation the direct method for determining overvoltage in a number of solutions at a nickel electrode has been used.
2. By adding concentrated solutions of various salts to sulfuric acid, sodium hydroxide, or water, the effect of the presence of salts on the overvoltage of solutions was determined.
3. In acid solution:
  - (a) The addition of a neutral salt produced a slight lowering of the overvoltage.
  - (b) The addition of a basic material caused an increase in the overvoltage followed by a decrease as the concentration was increased.
  - (c) Oxidizing agents caused the overvoltage to be lowered.
4. In sodium hydroxide solution:
  - (a) The addition of a neutral salt or salt of a weak acid caused a slight lowering of the overvoltage.
  - (b) The addition of sodium dichromate caused the overvoltage to rise followed by a lowering as the concentration was further increased.
5. At low concentrations of sulfuric acid, sodium hydroxide, or a salt in pure water, the overvoltage is very high. The value drops very rapidly at first and then more slowly as the concentration of the solute is increased.

# ANALYSIS OF CONTINUOUS SPACE FRAMES BY THREE-DIMENSIONAL MOMENT-DISTRIBUTION AND SLOPE-DEFLECTION METHODS<sup>1</sup>

SHIH-CHING LO

*From the Department of Civil Engineering, Iowa State College*

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Structural engineers have long been aware of the fact that structures very seldom lie in one plane as they are usually assumed in conventional method of analysis.

The author, as an eager student to learn has, however, made his effort to develop the two methods herein given to solve continuous space frames by taking the whole structure as a unit of analysis. The moment-distribution and slope-deflection methods which are generally used in solving planar continuous frames have been extended by the author to solve continuous space frames.

For the purpose of discussion, frames used in structures can be divided into two main classes, trussed frames and continuous frames. Trussed frames can be statically determinate or statically indeterminate. Continuous frames are, however, all statically indeterminate.

For the purposes of analysis continuous frames can be classified according to the number of possible directions of displacement: (1) no direction of displacement; (2) one direction of displacement; (3) two directions of displacement; (4) three directions of displacement.

The same assumptions have been used by the author as those upon which the original moment-distribution and slope-deflection methods have been based. Additional assumptions required for the continuous space frames are: (1) The effects of "warping" of the cross section and of the change of moment of inertia due to the shifting of the neutral axis caused by torsion are neglected; (2) the conditions of equilibrium which apply to the structure as a whole, to each joint, and to each member are:

$$\Sigma F_x = 0, \Sigma F_y = 0, \Sigma F_z = 0, \Sigma M_x = 0, \Sigma m_y = 0, \Sigma M_z = 0.$$

## I. THREE-DIMENSIONAL MOMENT-DISTRIBUTION METHOD

This method involves two distinct operations: First, to balance the moments and second, to balance the shears. To balance the moments at the joints, each joint is in a hinged-fixed condition which means that the joint is free to rotate but is prevented from linear displacement. This operation will set up unbalanced shears at each joint. To remove these unbalanced shears, the influence of the shear upon the moments on the whole frame must be determined.

In order to find the influence of a shear upon all the members in a

<sup>1</sup>Original thesis submitted June, 1941. Doctoral thesis number 637.

continuous space frame, the shear distribution method is developed. In applying this method a load is applied at one of the joints of the frame. The joints in line with the load are rigid-free, which means that the joints cannot rotate but can translate. All the other joints are considered for the time being as rigid-fixed, which means that they may neither rotate nor translate. Now the applied shear is distributed to each of the members adjacent to and perpendicular to the line of action of the applied shear. The amount of the applied shear carried by each member is proportional to the shear stiffness factors. These distributed shears are carried over to the other end of each member, where they are considered as newly applied shears and distributed as just outlined. By summing up these shears at the end of each member the corresponding bending moments can be computed at these ends.

When a unit shear is originally applied the moments thus obtained are called shear moment factors. Shear moment factors were devised by the author to correct the unbalanced shear somewhat similar to rectifying moments used in correcting the unbalanced shears in planar continuous frames. By alternately applying the two basic operations of balancing the moment and shears, the final solution will be obtained.

## II. THREE-DIMENSIONAL SLOPE-DEFLECTION METHOD

This method is developed with two purposes in mind: First, to show that the historical method of slope-deflection is applicable to the solution of a continuous space frame; and second, to introduce a check of the three-dimensional moment-distribution method for those who have greater confidence in the established slope-deflection equations.

In space frames, there are three possible rotations with respect to each of the axes of reference. In addition, the frame may have one, two, three, or no possible directions of displacement depending upon the nature of the problem. With the rotations and deflections defined, the end moments and torques can be expressed in terms of these values. Now the equilibrium condition of moments at any joint will give as many simultaneous equations as there are rotations. The rest should be supplied by the consideration of shears. If the change of length of the member is neglected, the deflections of all joints lying in a straight line will be equal. If there is no external load applied along this line, the shears developed along this direction by all contributing members should be equal to zero. When there is an external load applied at a joint along this line, then the shears should be equal to the external load. Thus one can obtain as many simultaneous equations as there are deflections. It is evident that necessary and sufficient numbers of simultaneous equations can be obtained, the solution of which needs no further description.

# THE ECOLOGY AND MANAGEMENT OF THE REDHEAD, NYROCA AMERICANA (EYTON), IN IOWA<sup>1</sup>

JESSOP BUDGE LOW

*From the Department of Zoology and Entomology, Iowa State College*

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An investigation of the Redhead, *Nyroca americana* (Eyton), continuing from March, 1938, to June, 1941, on the research area in Clay and Palo Alto counties had a threefold objective: (1) to determine the extent to which Redhead occurred and bred in Iowa, (2) to investigate further the life history and ecological relationships of this waterfowl species, and (3) to ascertain applicable management practices to improve the habitat of this bird.

The research area known as the "Ruthven Area," which consisted of six lakes and numerous marshes and sloughs, was located within 5 miles of Ruthven, Iowa. These water areas ranged from 1,200 acres in the largest to less than one-quarter of an acre in the smallest, an aggregate of approximately 5,800 acres.

Redhead reached the research area on their northern migration March 20, approximately. Peaks of spring migration were noted March 21-25 and April 11-18, the latter dates representing the main flight. Large open lakes were indispensable as resting, feeding, and refuge areas to the 3,000 to 4,000 Redhead observed on the marshes and lakes during the spring migration each year. Migrant Redhead preferred the open water of the lakes for resting at night, and bays of the lakes and large vegetation-covered marshes were frequented in the daytime.

The sex ratio was 1.42 males to 1 female among 3,400 Redhead.

Toward the latter part of April the mated ducks left the deeper water areas to seek nesting sites in the vegetation of the shallow marshes and sloughs. Marshes of 10 acres, or larger, situated not further than one-quarter mile from large permanent lakes were preferred nesting habitats. Although twenty-eight other species of marsh-nesting birds were observed in the marshes, there appeared to be no conflict between them and the Redhead. Numbers of early nesting Redhead as well as numbers of migrants were retarded by abnormally low temperatures during April and May, 1940. Nesting extended through 105 days, April 30 to August 12, 1940, a period of 28 days longer than in the two previous seasons. During the period May 8-24 the nesting season was at its height. Late re-nesting attempts, initiated after previous nests were deserted because of a decline in the water level, accounted for the extended 1940 nesting season.

A total of 160 nests were under observation: 42 in 1938, 53 in 1939, and 63 in 1940. Calculated on a 60 per cent nest find, these nests during

<sup>1</sup>Original thesis submitted June, 1941. Doctoral thesis number 620.

the average year on the 925 acres of vegetation available for nesting represented a density of 1 nest to 10.6 acres. A high density of one nest to 2 acres was found on each of several small potholes in Dewey's Pasture.

Ninety nests (56.25 per cent) terminated successfully. Twenty-two nests (13.75 per cent) were destroyed, and 48 nests (30.00 per cent) were deserted. A rising water level flooded twelve (54.55 per cent) of the unsuccessful nests, while mink destroyed four (18.18 per cent), and crows destroyed two (9.09 per cent) nests. Unknown agents destroyed four nests (18.18 per cent). The recession of the water level, particularly in 1940, was responsible for the desertion of sixteen nests (10.00 per cent), while a comparatively stable water level was correlated with the high percentage (73.57 per cent) of nest success in 1939. The apparent intolerance of the females to the laying of eggs in their nests by other ducks resulted in the desertion of sixteen nests (10.00 per cent). The amount of "promiscuous egg-laying" was inversely proportional to the nesting success and directly proportional to the fluctuation of the water level. The Ruddy Duck (*Erismatura jamaicensis rubida*) laid eggs in 8.12 per cent of the Redhead nests.

An average of 9.75 eggs were recorded for 115 complete clutches.

No definite rhythm of incubation was carried out by the female. An average of 17.5 hours a day was spent on the nest by one closely observed female during the 24-day incubation period. Hatching started June 1 and ended August 12 when the last nest, one of the sixteen re-nesting attempts, hatched. A total of 45.05 per cent of the eggs produced young. Undeveloped eggs, probably infertile, represented 4.68 per cent of the total eggs, while partially developed embryos left in the nest after normal hatching occupied 5.48 per cent of the eggs.

The most extensive nesting cover species were lake sedge (*Carex lacustris*) 23 per cent, narrow-leaved cattail (*Typha angustifolia*) 12 per cent, hardstem bulrush (*Scirpus acutus*) 10 per cent, and awned sedge (*Carex atherodes*) 10 per cent. The depth of the water in which the cover plants were located and proper interspersion and density of the nesting cover were more important criteria in the choice of nesting sites than was a preference which the ducks had for any cover plant species. Nesting densities for the important cover plants were as follows: one nest to 3 acres of slender bulrush (*Scirpus heterochaetus*), one nest to 6 acres of hardstem bulrush, one nest to 11 acres of whitetop (*Fluminea festucacea*), one nest to 13 acres of sedge and one nest to 16 acres of narrow-leaved cattail.

The nests were built in the marshes an average of 72 yards from the outer edge of the marsh vegetation, and 85 per cent of the nests were located within 50 yards of open water. The nearest open water for 64 per cent of the nests was around muskrat (*Ondatra zibethica*) lodges. Nesting densities reached a maximum where not less than 10 per cent and not more than 25 per cent of the habitat was open water. Nests were constructed over water which averaged 11 inches in depth.

The hardstem bulrush—river bulrush (*Scirpus fluviatilis*)—bur reed (*Sparganium eurycarpum*) community was most important as juvenile rearing cover. Brood counts showed that the greatest mortality to juveniles was during the first week of life. Mortality during the average year accounted for 28 per cent of the juveniles. Evidence was noted of juvenile mortality from mink (*Mustela vison*), snapping turtle (*Chelydra serpentina*), lead poisoning, exposure to weather, congenital disabilities, and trampling by the female or other young. The flight of the juveniles from the Ruthven Area preceded the fall migration of northern Redhead by nearly a month. An average of 6.6 juveniles (72 per cent) of each brood survived, which meant that 280 juveniles in 1938, 480 in 1939, and 200 in 1940, approximately, were reared in the research area. Leeches (*Theromyzon occidentalis*) parasitized 80 per cent of the young Redhead, but were not found to be a cause of death. Lead poisoning was not a serious cause of mortality either to juveniles or adults.

Redhead constituted approximately 5 per cent of the bagged birds checked in the research area during the 1939 shooting season. Cripple loss on all duck species was greatest in pass shooting (34 per cent) and least in shore-walking and jump-shooting (8 per cent). The need for additional public shooting grounds was evident from the large concentration of hunters on the limited public shooting grounds of the research area.

Food plants important to the Redhead were abundant in the research area.

Drainage, drought, and overshooting were the three principal causes for decline in numbers of Redhead within recent years. The cutting of wild hay was not injurious to Redhead production in Iowa marshes. Large marshes were not affected by moderate livestock grazing, although small marshes (under 25 acres) should be protected from livestock.

Improvement of the nesting habitat of the Redhead in Iowa must aim at the fulfillment of the needs of the bird, namely, water and emergent vegetation. As a means of habitat improvement it is suggested that dense blocks of unproductive nesting cover be opened up to provide an inter-spersion of small areas of water with the cover. An adequate stabilized water level is the most important factor to insure the production and utilization of cover and food plants.

The establishment and maintenance of an adequate water supply on some of the more unsuccessfully drained marshes and lakes in Iowa would not only be serviceable to the duck populations but would also serve as an important means of local control for two of Iowa's most annoying mosquitoes, *Aedes vexans* and *Aedes trivittatus*.

# ECOLOGY AND MANAGEMENT OF THE MOURNING DOVE, ZENAIDURA MACROURA (LINN.), IN SOUTHWEST IOWA<sup>1</sup>

H. ELLIOTT MCCLURE

From the Department of Zoology and Entomology, Iowa State College

Received June 10, 1941

The mourning dove, *Zenaidura macroura* (Linn.), was studied in southwestern Iowa at Lewis during 1938, 1939, and 1940 with special reference to its production, life history, and management. In 1938 and 1939 all of the nesting sites available to the birds were examined every other day in Lewis (an area of 160 acres), and on alternate days at fourteen farmyards and five miscellaneous habitats (an area of 60 acres). In 1940, in order to concentrate the work on a smaller area, 10 acres in town, three farmyards, and a state park were visited every day.

In the three years 2,876 separate nests were used 3,878 times by the doves. They laid 7,164 eggs and raised 3,396 young. In Cass county, towns make up 6 per cent of the area and produced 65 per cent of the birds, farmyards make up 1.6 per cent of the area and produced 20 per cent of the birds, while the remaining 92.4 per cent of the area produced only 15 per cent of the birds. The average number of young produced in Cass county each year was 228,000. Nesting success dropped from 58 per cent in 1938 to 37 per cent in 1940. This decrease was the result of increased weather loss and predator damage. Even though losses increased, an average of seven young per acre in town were raised successfully, the birds compensating for losses by additional nest building. Farmyard nesting success decreased constantly from loss of nesting facilities through destruction and death of trees.

Doves do not breed until a year after they leave the nest. The male carries sticks and grass to the female who is on a location that they have both agreed upon, and she builds the nest by crossing the sticks beneath her. Requirements of a good nest site include ease of approach and departure, visibility in all directions and concavity to support the nest. After building one or two days the first egg is laid, and on the following day the second is laid, but additional nest building continues through incubation, 14 days. Average height of nests was 20.2 feet, average trunk diameter of trees used was 17.4 inches, and the average distance from the center of the tree at which nests were built was 11.2 feet. Nests were built on all sides of trees, but preferably to the leeward of prevailing winds. Preferred nesting trees in town were American elm (*Ulmus americana*), box elder (*Acer negundo*), soft maple (*Acer saccharinum*), red pine (*Pinus resinosa*), apple (*Pyrus malus*), Norway spruce (*Picea abies*), tamarack (*Larix laricina*), and blue spruce (*Picea sp.*) in that order, and in the country red pine, apple, soft maple, Scotch pine (*Pinus*

<sup>1</sup>Original thesis submitted June, 1941. Doctoral thesis number 622.

*sylvestris*), Norway spruce, box elder, plum (*Prunus americanus*), and elm. Evergreens were used most heavily in the spring, but decreased in popularity as the deciduous trees came into leaf. Of the trees used, 1.1 per cent bore ten or more nestings.

Nests of the eastern robin (*Turdus migratorius*), bronzed grackle (*Quiscalus quiscula*), blue jay (*Cyanocitta cristata*), catbird (*Dumetella carolinensis*), brown thrasher (*Toxostoma rufum*), yellow and black-billed cuckoos (*Coccyzus americanus* and *C. erythrophthalmus*), English sparrow (*Passer domesticus*), pigeon (*Columba livia*), rose-breasted grosbeak (*Hedymeles ludovicianus*), and fox squirrel (*Sciurus niger*) supported 11.8 per cent of the dove nests. Seventy-eight per cent of these were placed in robin and grackle nests, with a success of 55 per cent.

The male dove incubates during the day, the female at night. Fifty-five per cent of the eggs hatched and 83 per cent of the young were successful in leaving the nest. The average length of juvenile life was 14.5 days, and an average of 1.82 young per nest was produced. The average number of nesting attempts per pair was six, with three successful broods.

Nests were often used again by the same or other pairs of doves, for 74.3 per cent were used once, 18.8 per cent twice, 5.3 per cent three times, 1.3 per cent four times, and .3 per cent five times. The number of renestings increased as the season progressed. Nest building during the season was divided into three phases: acceleration phase during which more nests were gained than lost each day, between March 31 and June 8; the fluctuation phase between June 9 and September 1, when daily losses were quickly regained by more nest building; and the deceleration phase from September 1 to the close of the season in October, when more nests were lost each day than gained.

At hatching young weighed between 5 and 6 grams, and when they left the nest they weighed from 70 to 75 grams, having increased in weight each day as much as their original weight at hatching. Juveniles reached a hundred grams by the age of 30 days. Adult weight was from 120 to 140 grams.

The greatest destructive agent to dove nests was weather, taking 22 per cent. The worse predators were blue jays, fox squirrels, cats (*Felis domestica*), and bronzed grackles in that order. Nests were subject to losses from many other causes. Forty-nine per cent of nest losses were of indeterminable origin.

Young were fed pigeon milk and seeds regurgitated from the crops of both parents. Young instinctively know how to drink and fly but at weaning must learn to eat. Examination of the crops of young doves killed by accidents revealed a diet including the seeds of forty-four species of plants. Hemp (*Cannabis sativa*) ranked first in appearance in crops and by weight and volume. Green foxtail (*Setaria viridis*) ranked second in appearance and in numbers of seeds eaten. Yellow foxtail (*S. glauca*) ranked third in appearance and by volume and weight. Eleven other species making up more than 1 per cent by number, volume, and weight

were prostrate pigweed (*Amaranthus blitoides*), pigweed (*A. retroflexus*), ragweed (*Ambrosia artemisiifolia*), sorrel (*Oxalis stricta*), the spурges (*Euphorbia preslii*, *E. heterophylla*, and *E. maculata*), corn (*Zea mays*), wheat (*Triticum sativum*), sudan grass (*Sorghum sudanensis*), and hegari (*S. vulgare*). Seventeen species of land snails and six species of aquatic snails were fed to the young in order to fill their calcium requirements. Forty-four per cent of these snails were of the genus *Gastrocopta*. Grit is essential, and doves eat from 50 to 100 pebbles a day.

Doves make considerable use of the voice and have a number of different calls, commonest of which is the male's coo. Cooing activity was affected by the weather, temperature, and wind. There was less cooing during cloudy weather, high winds, and high temperature. It was greatest at morning and evening.

Doves were occasionally parasitized by the hippoboscid (*Microlynchia pusilla*) the mallophagans *Menopon* sp., and *Columbicola columbae*, and the feather mite *Liponyssus sylviarum*.

Young were banded between the ages of 4 and 9 days. Twelve returns from 1,600 banded birds showed their migration from Iowa into Texas, Mexico, and Central America. Two per cent of the dove population over-winters in Iowa.

Management suggestions for the maintenance and increase of doves in southwestern Iowa includes the planting of American elms and evergreens in small towns. Management suggestions for the farm are to increase the acreage of orchards, to plant more evergreen windbreaks, and to refrain from cutting over or overgrazing woodlands and gullies.

# BIONOMICS AND MORPHOLOGY OF THE BLACK ONION FLY, TRITOXA FLEXA (WIED.) (DIPTERA, ORTALIDAE)<sup>1</sup>

HUBERT CLYDE MANIS

From the Department of Zoology and Entomology, Iowa State College

Received June 10, 1941

The black onion fly, *Tritoxa flexa* (Wied.) is potentially a very serious enemy to onion growers throughout the North Central and New England States. The first account of it as an economic species is that given by Dr. Henry Shimer in "The Prairie Farmer" in 1865. Since that time it has been reported from Connecticut, New Jersey, Pennsylvania, Ohio, Indiana, Wisconsin, Minnesota, California, and Iowa. In Iowa it is as serious a pest of onions as the onion maggot, *Hylemyia antiqua* Meig., in the onion-growing localities of Pleasant Valley, St. Ansgar, and Clear Lake.

Laboratory rearing methods were quite simple. The adults were fed a mixture of malt, yeast, honey, and water and were kept in large screen cages containing growing onions which provided a place for egg deposition. The larvae, for life history studies, were reared individually in onion bulbs placed in small, clear glass jars with screw-top lids.

The black onion fly is a moderately large black fly with three oblique, hyaline crossbands on the wings, and is readily distinguished from all other species of flies which infest onions. The adult male and female of *T. flexa* are very much alike in appearance except in the region of the abdomen. In the male the abdomen is plump and more rounded with only 4 abdominal segments visible from above, and the genital complex is not bilaterally symmetrical. In the female the abdomen is much more slender with five abdominal segments visible from above and the sixth segment distinctly flattened dorso-ventrally. The genital complex of the female consists of three segments and is bilaterally symmetrical.

The white, semipolished egg is cylindrical, tapering gradually to the cephalic end to form a distinct micropyle. The egg is distinctly curved, and the surface sculpturing forms an irregular netlike pattern. The average length of the egg is 1.40 mm. and the average width is 0.30 mm.

The larva is similar in shape to the general dipterous type larva, but can usually be distinguished from the larva of other onion maggots by the profile of the last segment, which is more squarely truncate, and by the absence of prominent tubercles around the margin of the anal field. The larval instars can be readily separated by differences in morphology. The great hooks of the first instar are bidentate, and the parastomal sclerites are absent. The first instar does not possess anterior spiracles, and the posterior stigmal plate bears a single v-shaped spiracular opening. The great hooks of the second instar are also bidentate, but the parastomal sclerites and the anterior spiracles are present. The stigmal plate bears

<sup>1</sup>Original thesis submitted June, 1941. Doctoral thesis number 628.

three spiracular openings and a button. The great hooks of the third instar are not bidentate, and like the second instar, the parastomal sclerites and anterior spiracles are present. The stigmal plate is, in general, like the stigmal plate of the second instar, but is more heavily chitinized.

The puparium is a typical dipterous type pupal case, reddish brown in color, and retains many of the external characters of the third instar larva. Its average length is 6.65 mm.

The adults begin to emerge in May and apparently feed on the nectar of flowers and other plants in the fields. The female usually lives longer than the male. Copulation most often occurs in the late afternoon when the flies are congregated on weeds and grasses around the margins of the fields. One female may mate as high as seven times during the oviposition period and it is not uncommon for her to mate more than once before ovipositing. The preoviposition period is usually about two weeks in length, but this period varies considerably.

Oviposition usually occurs in the early morning or late afternoon. The eggs are deposited singly or in masses in the soil about the base of the plant, on the leaves and stems, and between the leaf sheath and the stem. The highest number of eggs laid by a single female was 109. The eggs hatch in about 4 days, and the young larvae begin to feed almost immediately. Development during the first and second stadia is quite rapid and requires approximately 10 days. Development during the third stadium is much slower since this stadium is more than two weeks in length. Larvae migrate very little except in search of food and just prior to pupation. When the larvae become fully mature they nearly always leave the bulb and burrow into the soil for a short distance before pupating. The puparium is usually found about 3 inches under the soil and close to the infested bulb. The pupal stage lasts approximately two weeks. There are two and a partial third generations a year in Iowa. Because of the long oviposition period and the wide range in the length of larval development there is an overlapping of generations. All stages of the life cycle can be found in the fields at the same time during the summer months. The biggest percentage of maggots overwinter as partially grown larvae in the onion bulb, although some of them pupate and pass the winter in that stage in the soil. The sex ratio of reared individuals was approximately 50-50.

The known host plants of *T. flexa* are limited to cultivated varieties of onions and chives. Since this insect is native it undoubtedly will feed on species of wild onions. It shows a preference for the yellow bottleneck and white Bermuda onion when these are available.

In Iowa, in the field, most of the economic damage is done to young seedling onions. This is due to the fact that a single maggot can destroy several such plants before reaching maturity. Only under conditions of extremely heavy infestation will there be much loss to set onions. When heavily infested, the plants are completely destroyed, leaving only the tops and outer husks of the bulbs.

The only parasite found attacking *T. flexa* was an undetermined species of fungus, which occasionally attacks the larvae. This insect may have a number of parasites but none were ever reared from larvae or adults collected in the field nor does literature record any. Many species of spiders prey upon the adults and probably many predaceous insects attack them in the field, although none were observed to do so.

Many species of flies were found associated with *T. flexa* in the field. The most common are *Tritoxa incurva* Loew., the onion maggot (*Hylemyia antiqua* Meig.), the seed corn maggot (*Hylemyia cilicrura* Rond.), the barred wing onion fly (*Chaetopsis aenea* Wied.), the spotted root fly or cantaloupe fly (*Euxesta notata* (Wied.) ), the lesser bulb flies (*Eumerus strigatus* Fall. and *Syritta pipiens* L.), and the stratiomyid (*Psecticus trivittatus* Say).

# CONDENSATION OF MERCAPTANS WITH CHLORAL IN THE GASEOUS PHASE<sup>1</sup>

FRANCIS B. MOORE

*From the Department of Chemistry, Iowa State College*

Received May 22, 1941

There are two opposing opinions as to the effect of the solvent upon the numerical value for the dissociation constant of a substance. According to one viewpoint<sup>2</sup> the effect is additive and predictable, while from another<sup>3</sup> it is irregular and the gaseous phase dissociations furnish the true measure of the equilibrium.

The electron-sharing ability of an organic radical<sup>4</sup> is a measure of its influence upon the polar characteristics of its compounds. If the solvent effect upon the dissociation constant is large, the constant determined in solution would not show the same relationship to the electron-sharing ability as that determined in the gaseous phase. A comparison of results obtained in both states is therefore of great interest and is the purpose of this investigation.

Chloral was condensed with methyl, ethyl, butyl, and allyl mercaptan in the gaseous phase to form the corresponding hemimercaptals. The dissociation constants for the reaction



were calculated from the pressures of the three constituents in the equilibrium mixture.

Ethyl and methyl mercaptan were obtained from the Eastman Kodak Company and fractionally distilled. Their boiling points were 36° and 6.7°, respectively. Butyl mercaptan, boiling point 97°, was prepared by the method of Ellis and Reid<sup>5</sup>. Allyl mercaptan, boiling point 67-68°, was prepared by the method of Braun and Murjahn<sup>6</sup>. Chloral was prepared from chloral hydrate by dehydrating it with concentrated H<sub>2</sub>SO<sub>4</sub>. The fraction boiling at 97° was used.

The apparatus used in these determinations consisted of a breaking device for liberating equimolar quantities of chloral and mercaptan and a trace of dry HCl into a reaction flask of 1,130 ml. capacity. After each constituent had been released its pressure was balanced by admitting dry nitrogen to a balance flask. The condition of balance was determined by means of a new type of balance gauge consisting of a mercury manometer

<sup>1</sup>Original thesis submitted August 21, 1940. Doctoral thesis number 593.

<sup>2</sup>Goodhue and Hixon, *J. Am. Chem. Soc.*, **56**, 1329 (1934).

<sup>3</sup>Conant, *Ind. Eng. Chem.*, **24**, 466 (1932).

<sup>4</sup>Hixon and Johns, *J. Am. Chem. Soc.*, **49**, 1786 (1927).

<sup>5</sup>Ellis and Reid, *J. Am. Chem. Soc.*, **54**, 1674 (1932).

<sup>6</sup>Braun and Murjahn, *Ber.*, **59B**, 1202 (1926).

TABLE I  
THE DISSOCIATION CONSTANTS OF A SERIES OF HEMIMERCAPTOALS IN THE GASEOUS PHASE

$M \times 10^4$ RSH	$M \times 10^4$ Chloral	$P_{eh}$ (mm)	$P_{ash}^*$ (mm)	$P_{total}^{initial}$ (mm)	$P_{total}^{final}$ (mm)	$P_{ash}$ final (mm)	$K_p$ (atm.) (25°C.)
CH <sub>3</sub> SH							
4.102	4.089	0.587	0.617	1.204	1.013	0.191	$1.16 \times 10^{-4}$
4.238	4.083	0.667	0.696	1.363	1.152	0.211	$1.38 \times 10^{-4}$
0.898	0.980	0.125	0.116	0.241	0.228	0.013	$1.17 \times 10^{-4}$
C <sub>2</sub> H <sub>5</sub> SH							
2.290	2.311	0.340	0.345	0.685	0.372	0.313	$3.63 \times 10^{-4}$
1.169	1.200	0.162	0.163	0.325	0.228	0.097	$5.82 \times 10^{-5}$
0.887	0.826	0.120	0.131	0.251	0.184	0.067	$6.86 \times 10^{-5}$
CH <sub>2</sub> SH							
0.877	0.985	0.139	0.136	0.275	0.217	0.058	$0.032$
6.769	6.598	0.982	1.071	2.053	1.282	0.771	$0.065$
C <sub>3</sub> H <sub>7</sub> SH							
1.984	2.145	0.288	0.280	0.578	0.443	0.135	$0.145$
2.799	2.885	0.419	0.421	0.840	0.520	0.320	$2.30 \times 10^{-4}$
1.547	1.519	0.226	0.226	0.452	0.341	0.111	$4.11 \times 10^{-4}$
						0.115	$1.57 \times 10^{-4}$

\* This value was read from the gauge as follows: the deflection produced by the chloral was measured 5 minutes after the compound had been released. The deflection produced by the RSH compound was similarly measured. From the ratio of these deflections and the measured pressure of the chloral, the pressure of the RSH compound was calculated.

† This value is the pressure due to the hemimercaptal formed.

‡ These values were discarded because the equilibrium was not homogeneous.

of large area cross-section in conjunction with a horizontal capillary tube. Dicyclohexyl extended from one mercury surface into the capillary tube, and this meniscus registered any difference in pressure between the reaction and balance flasks. The gauge was sensitive to differences of 0.001 mm. and could be used for pressure differences up to 2 mm. When balance had been established the pressure was read by means of a McLeod gauge.

The results obtained in these experiments are shown in Table 1. In columns 1 and 2 are shown the number of moles of mercaptan and chloral released. Columns 3 and 4 give the pressure produced by the release of the chloral and mercaptan. The pressure when equilibrium had been established is given in column 6. Columns 7, 8, and 9 give the pressures of the three constituents in the equilibrium mixture from which the dissociation constant given in column 10 was calculated. It was noted that condensation of butyl hemimercaptal occurred at a pressure of 0.685 mm., and allyl hemimercaptal at 0.840 mm.

The free energies of dissociation in benzene and in the gaseous phase were calculated using the relationship  $\Delta F^\circ = -RT\ln K$  and are shown in

TABLE 2  
FREE ENERGIES OF HEMIMERCAPTALS IN BENZENE AND THE GASEOUS PHASE

Hemi-mercaptal	$K_{dissoc}$ Benzene	$K_x$ Benzene	$K_{atm}$ Gaseous	$\Delta F^\circ$ Cal. Benzene	$\Delta F^\circ$ Cal. Gaseous
n-C <sub>4</sub> H <sub>9</sub>	$2.0 \times 10^{-3}$	$1.5 \times 10^{-4}$	$6.24 \times 10^{-5}$	5.2	5.7
C <sub>2</sub> H <sub>5</sub>	$2.35 \times 10^{-3}$	$? 1 \times 10^{-4}$	$1.24 \times 10^{-2}$	5.0	4.0
CH <sub>3</sub>			$1.26 \times 10^{-4}$		5.3
C <sub>3</sub> H <sub>7</sub>			$1.94 \times 10^{-4}$		5.1
C <sub>4</sub> H <sub>9</sub> CH <sub>3</sub>	$2.8 \times 10^{-3}$	$2.4 \times 10^{-4}$		4.9	
p-CH <sub>3</sub> C <sub>6</sub> H <sub>4</sub>	$1.55 \times 10^{-3}$	$1.2 \times 10^{-3}$		4.0	
C <sub>6</sub> H <sub>5</sub>	$2.1 \times 10^{-2}$	$1.63 \times 10^{-3}$		3.8	
p-ClC <sub>6</sub> H <sub>4</sub>	$4.1 \times 10^{-2}$	$3.2 \times 10^{-3}$		3.4	

Table 2. Column 1 gives the values previously determined in this laboratory<sup>7</sup>. Column 2 shows these values recalculated in terms of mole fractions. Column 3 gives the average values obtained from Table 1. Columns 4 and 5 show the free energy changes calculated from columns 2 and 3.

With the exception of the value for the ethyl hemimercaptal, the trend in the gaseous phase seems to differ little from that in the benzene solution, indicating but little solvent effect. It seems probable that no significant differences exist between the results obtained in the two phases, and that the electron-sharing abilities of the radicals are the same in benzene solution and the gaseous phase. It is evident that the effect of the benzene solvent upon the value of  $\Delta F^\circ$  for these dissociations is slight. Since the measurement of gaseous equilibria at low pressures is

<sup>7</sup> Johns and Hixon, J. Am. Chem. Soc., 56, 1333 (1934).

tedious and subject to experimental error, there is no advantage to be gained in this case.

Equilibrium between gaseous chloral, mercaptan, and hemimercaptal was established in from 24 to 72 hours. The dry HCl catalyst which is so essential when the reactions are run in benzene solution has no apparent effect upon the reactions in the gaseous phase. Equilibrium was established at the same velocity when it was absent as when it was present.

# RELATIONSHIP BETWEEN THE ELECTRON-SHARING ABILITY OF RADICALS AND THE IONIZING POWER OF NON-AQUEOUS SOLVENTS<sup>1</sup>

FRANK J. MOORE

From the Department of Chemistry, Iowa State College

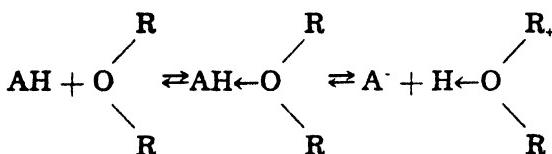
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The main factors affecting ionization of a given acid in a solvent are:

- (1) The basicity of the solvent (its ability to accept protons).
- (2) The dielectric constant of the solvent.

In a general way, the greater the dielectric constant of a solvent, the greater is its ionizing power. This has long been known, and as a consequence the latter of the above two factors is commonly regarded as determining the ionizing power of a solvent. However, many exceptions to this rule have been noted, particularly with solutions in non-aqueous solvents.

The following reaction is written to illustrate the ionization of an acid in a non-aqueous solvent, taking an ether as example.



It has been shown that ionization of this type is dependent on preliminary compound formation between solvent and solute, as shown above, and that the degree of ionization increases as the extent of intermediate compound formation increases. Hunt and Briscoe<sup>2</sup> interpreted this as being due to the formation of coordinate-covalent bonds between the hydrogen of the acid and a donor atom in the solvent molecule. Further, they stated that the ionization of the acid should be dependent on the electronic properties of the groups attached to the donor atom in the solvent molecule. If these were "electropositive" groups, enhancing the donor power of the donor atom, intermediate compound formation and ionization should be relatively large. The opposite effect was predicted for "electronegative" groups.

The electron-sharing ability (abbreviation, E.S.A.) of a radical may be visualized as its tendency to attract or release the electrons in the bond uniting it with another atom in a chemical compound, relative to the tendency of hydrogen, attached in the same way, to attract or release these electrons. Radicals of large E.S.A. tend to attract electrons; radicals of small E.S.A. tend to release them.

<sup>1</sup>Original thesis submitted December 18, 1940. Doctoral thesis number 597.

<sup>2</sup>Hunt and Briscoe. *J. Chem. Ed.* 6:1716 (1929).

Returning to the example above, it may be seen that if R is a radical of large E.S.A., it will attract electrons toward itself, tightening the electron octet of the oxygen atom and restricting its donor power. This means that the extent of intermediate compound formation (and hence ionization) will be relatively slight. Radicals of small E.S.A. should have an opposite effect.

The solvents chosen for this investigation had nearly equal dielectric constants, so that variations in the extent of ionization of picric acid in the solvents should depend solely on their basicities. Both the cryoscopic and conductimetric methods were used for measuring the ionization.

For the cryoscopic measurements a series of ketones of the type  $\text{RCOC}_6\text{H}_5$  was employed. Picric acid was the solute in all cases. The usual cryoscopic apparatus was used to make the measurements, and the degree of dissociation was calculated from the apparent molecular weight.

Results are given in Table 1. Although runs were made for a series of solutions in each solvent, data are presented for only two concentrations (mole fraction of picric acid) to avoid multiplicity of examples.

TABLE 1  
IONIZATION OF PICRIC ACID IN KETONES  $\text{RCOC}_6\text{H}_5$ .

Substance	Dielectric Constant	E.S.A. of R	Per Cent 0.012 N <sub>A</sub>	Ionized 0.010 N <sub>A</sub>
Propiophenone .....	15.5 at 17°	-1.80	79	83
Acetophenone .....	18.1 at 20°	-1.45	73	76
Phenyl p-tolyl ketone .....	None reported	2.90	28	35
Benzophenone .....	12.4 at 40° 11.4 at 60°	4.20	13	19

Ionization was determined in other solutions by the conductance method, which is superior in many ways to the cryoscopic method. The solvents were ketones ( $\text{RCOCH}_3$ ) and nitriles ( $\text{RCN}$ ). The solute was picric acid in all cases. Results are given in Table 2.

Results obtained by both methods bear out the predictions made in the previous part of this paper. As the E.S.A. of R increases, the ionizing power of the solvent decreases. The dielectric constant is apparently of secondary importance in determining the extent to which these solvents ionize picric acid.

TABLE 2  
IONIZATION CONSTANTS FOR PICRIC ACID IN NON-AQUEOUS SOLVENTS AT 25°C

Substance	Dielectric Constant	E.S.A. of R	Ionization Constant
Methyl ethyl ketone .....	17.8 at 20°	-1.80	$1.98 \times 10^{-4}$
Acetone .....	21.5 at 20°	-1.45	$1.38 \times 10^{-4}$
Acetophenone .....	18.1 at 20°	4.20	$1.50 \times 10^{-5}$
Propionitrile .....	27.0 at 20°	-1.80	$4.25 \times 10^{-4}$
Benzonitrile .....	26.5 at 20°	4.20	$1.02 \times 10^{-4}$

Association of the solvent has a profound effect on the rate at which ionization takes place. Some of these solvents possess methylene groups, the hydrogen of which is replaceable by metal. The work of Landee<sup>3</sup> has shown that compounds of this type can associate by hydrogen bridge formation.

The maximum covalence of oxygen is believed to be three. Therefore, when molecules containing this atom associate, the donor power of the oxygen atoms is used up in forming the association bond, and these atoms cannot solvate the acid. Thus, if the dissociation of associated molecules into single molecules (able to solvate the acid) is slow, this might well be the controlling rate in the ionization process. Something of the sort appears to be the case. In those solvents where association was possible, ionization was always slow, requiring sometimes more than three days to reach equilibrium. Where association was not possible, three hours was sufficient for this result.

This indicates a possible connection between the association of a solvent and its ionizing power. It has long been recognized that solvents possessing fair degrees of ionizing power were associated in the liquid state. The fact that the solvent is associated indicates the presence in its molecule of atoms of considerable donor power, and as was shown in the work discussed above, the ionization of acids is dependent on this same property. If the assumptions made above, regarding the solvating power of the association complex, are valid, then the ionizing power of such solvents must not be due to their association, but rather in spite of it.

<sup>3</sup>Landee, Doctoral thesis number 504. Library, Iowa State College (1938) [Unpublished].

# SOME METAL-METAL INTERCONVERSIONS WITH ORGANOLITHIUM COMPOUNDS<sup>1</sup>

FRED WARREN MOORE

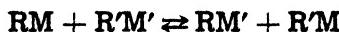
From the Department of Chemistry, Iowa State College

Received May 22, 1941

Organolithium compounds have become important aids in organic syntheses and have been used in many types of reaction. A survey of these reactions was presented with special emphasis being given the many different reactions these compounds undergo. The investigation of metal-metal interconversion reactions with organolithium compounds was made to increase the present knowledge of this useful reaction and to correlate the relative reactivities of organometallic compounds in this reaction with the reactivities observed in other types of reaction.

A study of the preparation of organolithium compounds by the reaction of organic halides with lithium metal in solvents other than diethyl ether was made. It was found that low-boiling petroleum ether (b.p. 28-38°) was a very satisfactory solvent for the preparation of the lower alkyl-lithium compounds with the exception of methylolithium. Yields were higher than those obtained using diethyl ether as solvent, especially in the cases of iso-propyllithium and s-butyllithium. The use of low-boiling petroleum ether as a solvent permitted the preparation of a new organo-lithium compound, t-butyllithium. The series of saturated alkylolithium compounds in which the metal atom is attached to a primary, to a secondary, and to a tertiary carbon atom, respectively, is now complete. In addition to the higher yields obtained, other advantages were found in the use of this solvent. Its low boiling point facilitated its removal and replacement by other solvents. The fact that it is unaffected by organo-lithium compounds, in contrast to diethyl ether, allowed the preservation of stock solutions which were found to be unchanged after long periods. Filtration, using a simple, easily constructable apparatus, removed inorganic by-products. The resulting clear, water-white solutions of organo-lithium compounds were useful in many reactions where the presence of excess lithium metal and inorganic salts was undesirable.

The metal-metal interconversion reaction is apparently quite general. The reaction, in general form, may be written as follows:



The extent and direction of this general reaction is determined by several factors. Some of the more important of these were found to be the solvent used, the nature of the organometallic compound and the time allowed for reaction to take place. Moderate temperature changes were found to have little effect.

<sup>1</sup> Original thesis submitted March 13, 1941. Doctoral thesis number 602.

With a given pair of organometallic compounds the most important factor was found to be the solvent used. Thus, with tetraphenyllead and *n*-butyllithium in diethyl ether, reaction to form tetra-*n*-butyllead and phenyllithium occurred to the extent of 70 per cent. Using benzene as solvent, only a slight interconversion was noted, whereas in petroleum ether no interconversion could be detected. In a similar manner the interconversion reaction between tetra-*p*-chlorophenyltin and *n*-butyllithium in diethyl ether took place to the extent of 72 per cent in 10 minutes, but in benzene-petroleum ether only 40 per cent interconversion had taken place in 4 hours.

The nature of the organometallic compounds was found to be an important factor, also. The reaction of tetraphenyllead with alkylolithium compounds in diethyl ether solution showed the following order of decreasing rates of interconversion: ethyl, *n*-propyl, *n*-butyl, methyl. With tetra-*p*-chlorophenyltin and the isomeric butyllithium compounds in petroleum ether the order of decreasing rates of interconversion obtained was: *n*-butyl = iso-butyl = *s*-butyl > *t*-butyl. The reaction of various organometallic compounds with *n*-butyllithium in diethyl ether showed the following order of decreasing rates of interconversion: tetraphenyltin, tetraphenyllead, tetra-*o*-tolyllead.

The tendency of radicals attached to metals like tin, lead, and mercury to be cleaved and become attached to lithium in the metal-metal interconversion reaction was found to decrease in the following order: *p*-chlorophenyl, *p*-tolyl, phenyl, *o*-tolyl.

These results indicated that the order of cleavage of radicals from organometallic compounds was dependent on several factors other than the nature of the radical. Such factors as the solvent used, the nature of the metallic atom holding the radical, and the nature of the cleaving agent were found to play important roles. The electronic configuration of the radical is not the only characteristic determining its order of cleavage. The order is somewhat dependent on the spatial configuration, also, as was shown by the low rate of interconversion of tetra-*o*-tolyllead by *n*-butyllithium and of diphenylmercury and dibenzylmercury by *t*-butyllithium.

Correlations of the relative reactivities of organometallic compounds in metal-metal interconversion reactions with observed reactivities in other types of reaction, like halogen-metal interconversion and hydrogen-metal interconversion, (metalation) were not possible in all cases. This may be due, in part, to side reactions which take place in many of these reactions. Among these may be mentioned the cleavage of solvents like diethyl ether by the organolithium compounds and coupling reactions in halogen-metal interconversion reactions.

# THE CONCENTRATION, CHARACTERIZATION, AND PROPERTIES OF SOYBEAN AMYLASE<sup>1</sup>

J. MARSHALL NEWTON

*From the Department of Chemistry, Iowa State College*

Received June 10, 1941

Preliminary investigations indicated that the soybean is a good source of an enzyme which will hydrolyze starch to reducing sugars. The enzyme content of the soybeans was found to differ from variety to variety. A difference in the amylase content of the same variety from year to year is also indicated. Germination of the soybeans under conditions which reduced the bacterial growth to a minimum produced a small decrease in the amylolytic activity of the beans. The decrease in the total amylase content during germination is in contrast to the marked increase produced by germination of most seeds. The increase is generally explained as due to the formation or liberation of  $\alpha$ -amylase during the germination. For some reason the soybean lacks this ability to produce or liberate  $\alpha$ -amylase during the sprouting process.

Fractional precipitation of alcoholic extracts of the soybean yield concentrates with high amylolytic activity. A maximum concentration of 225-fold has been obtained by this method. The most highly active concentrate acting on a gelatinized starch was capable of producing 4,580 times its weight of maltose in 30 minutes at 40° C. When allowed to act on soluble starch, 0.033 milligrams of this concentrate converted 10 grams of the gelatinized starch to maltose (60 per cent) and limit dextrin (40 per cent) in 36 hours at 40° C. This corresponds to a saccharogenic activity of 181,000.

The application of the concentration procedure to the low-activity enzyme concentrates gave products of considerably higher activity. The recovery of the enzyme in three concentration experiments was 63.2 per cent, 60.8 per cent and 68.0 per cent. By re-working such low-activity concentrates, the amylase concentration has been increased as much as tenfold in one operation. Since a large portion of the total amylolytic enzyme extracted from soybeans remains in the low-activity concentrates, a method for their further concentration is of much value.

The classification of the amylolytic enzyme of soybeans as  $\beta$ -amylase rests on a series of measurements and observations which are considered as indicative of the amylase type. Characterization of the enzyme by the diffusion method of Wijsman, determination of the amyloclastic power of the concentrates, the mutarotation of the starch degradation products, the isolation of maltose and a residual dextrin as the starch degradation products, the viscosity changes of the starch substrate during digestion, and the iodine-color reaction of the degradation products characterize the

<sup>1</sup>Original thesis submitted June, 1941. Doctoral thesis number 605.

starch-hydrolizing enzyme of soybeans as  $\beta$ -amylase. The only evidence which points toward the presence of  $\alpha$ -amylase in the concentrates is the marked ability of the enzyme to reduce the relative viscosity of the starch pastes in the early stages of the hydrolysis. However, since soybean amylase is one of the most powerful sugar-forming enzymes, this sugar coming from the hydrolysis of the starch substrate, it is not disturbing to observe a marked decrease in the relative viscosity of the substrate during digestion by  $\beta$ -amylase.

The purified soybean  $\beta$ -amylase concentrates were very stable. A dry concentrate with a saccharogenic activity of 600 showed no decrease in activity over a storage period of twenty-six months at 5° C. Aqueous solutions of the soybean  $\beta$ -amylase were also remarkably stable, this stability being proportional to the purity of the concentrates. A concentrate with a saccharogenic activity of 4,580 retained 87 per cent of its activity after 34 days in an aqueous solution maintained at 24-28° C.

Considerable evidence as to the nature of the products formed from starch by the action of soybean  $\beta$ -amylase is available from the literature. In order to compare the action of the  $\beta$ -amylase with the action of  $\alpha$ -amylase, it was necessary to have some idea of the nature of the products formed by the action of the latter enzyme on starch. With this objective in mind, the fractionation of the degradation products formed by the action of  $\alpha$ -amylase on starch and the characterization of the products by physical and chemical means were carried out.

The physical and chemical properties of the various fractions of the degradation products isolated from the digestion of cornstarch by  $\alpha$ -amylase indicate that the materials range from highly polymerized insoluble polysaccharides to very soluble di- and tri-saccharides. Attempts to separate these digestion products into definite chemical identities were not successful. In the various purification procedures, fractions with similar properties were obtained, but further fractionation gave a series of dextrans with a range of properties.

A highly insoluble polysaccharide was isolated from the  $\alpha$ -amylase digestions of cornstarch in about 5 per cent yields. This water-insoluble fraction resembles the insoluble material which forms in  $\beta$ -amylase digestions. However, the  $\alpha$ -amylase product does not flocculate, is more insoluble, and is obtained in higher yields than is the  $\beta$ -amylase product. Further investigations of the  $\alpha$ -amylase product are needed to point out the similarities and differences between the water-insoluble materials produced by the two amylases.

The fractionation of the degradation products formed by the action of  $\alpha$ -amylase on waxy maize starch was more readily accomplished than with the cornstarch degradation products. Although fractionation was not complete, the properties of certain of the products indicate that they consist primarily of the same, or very similar, materials.

Four corn dextrans and three waxy maize dextrans were selected as typical fractions and were subjected to physical and chemical measurements. The molecular weights of the dextrans were calculated from the

freezing-point depressions of the dextrans in water, the freezing-point depressions of the acetates in benzene, the reducing equivalent of the dextrans and from the iodine and potassium hydroxide consumption in the Kline and Acree method for the determination of aldose sugars. The molecular weights calculated from the reducing equivalent, the iodine and potassium hydroxide consumption, and the freezing-point depressions of the acetates agree quite satisfactorily in most cases. In contrast, the molecular weights as calculated from the freezing-point depression of the dextrans in aqueous solution are much below the values obtained from the other data. However, the abnormal freezing-point depression of dextrans and similar polyhydroxy compounds is the rule rather than the exception.

The potassium salt of the acid formed by the oxidation of one of the corn dextrans was isolated and the potassium content determined. From the potassium analysis the calculated molecular weights of the salts were 913 and 901, which gives corresponding values of 875 and 863 for the molecular weight of the original dextrin. This compares to a value of 880 calculated from the iodine consumed in the oxidation. This indicates that the aldehyde groups in the dextrans were oxidized to the corresponding acid, the acid then being neutralized by the potassium hydroxide to give the potassium salt.

A further aid in characterization of the dextrans and dextrin acetates was furnished by the melting points of the dextrans and dextrin acetates. Although the melting points were not sharp, often varying over a 10-20° range, they served to prevent the identification of some of the fractions as known simple sugars or their derivatives.

A study of the reaction of some of the low molecular weight fractions with liquid phenylhydrazine shows that compounds are not formed. Repeated precipitation of the products from various solvents yields a bright-yellow, amorphous product. However, all of the color and the nitrogen were removed from an aqueous solution of the products by adsorbent charcoal. The dextrinous material recovered after adsorption of the color was very similar to the original dextrin.

# QUANTITATIVE VOLUMETRIC TITRATION OF HEAVY METALS

HOWARD FREDERIC NICOLAYSEN

*From the Department of Chemistry, Iowa State College*

Received June 10, 1941

A great many precipitates of metals are known which should be easily oxidized. Quite a number of these involve organic precipitants.

The purpose of this research was to see if various precipitates of nickel and cobalt could be oxidized quantitatively. Such oxidations might serve as bases for quantitative volumetric determinations of these metals. Studies were made of the compounds of nickel with dimethylglyoxime, cobalt with alpha-nitroso-beta-naphthol, cobaltous tetrapyridine thiocyanate and the triple nitrite of potassium, cobalt, and lead.

Oxidation of alpha-nitroso-beta-naphthol was attempted first. Potassium permanganate was used as the oxidizing agent in both acid and alkaline solutions. Various concentrations of acid or base and of permanganate were used. The samples were allowed to stand after the reagents were mixed. Some samples were heated to boiling, and some left cold.

The results of these trials revealed greater oxidation in acid solution. More consistent data were obtained by using alkaline solutions. A strongly alkaline solution and a great excess of permanganate were found necessary. Little difference was noted in results whether the solution was boiled or left cold.

With these conditions maintained, direct oxidation of the cobalt compound was attempted. No success was had in these experiments.

Indirect determination of cobalt was attempted by precipitating with known excesses of a solution of the naphthol in 50 per cent acetic acid. Most of the curve obtained by plotting the permanganate requirement against the volume of naphthol was approximately linear. This fact indicated the possibility of determining limited quantities of cobalt.

Blanks on the acetic acid contained in the naphthol solution demonstrated the necessity of correcting the permanganate requirement. Subtraction of the amounts required for the blanks gave poor results. When a series of samples containing a constant volume of acetic acid were oxidized, proportionality between the naphthol and permanganate was indicated.

Small amounts of cobalt were then precipitated with constant amounts of naphthol, and the excess of the latter oxidized. A straight line relation between the amounts of cobalt and permanganate was found.

It was concluded that small amounts of cobalt could be determined in a strongly alkaline solution in the presence of a large excess of permanganate.

<sup>1</sup> Original thesis submitted June, 1941. Doctoral thesis number 621.

The limitation of amount determinable and the fact that the determination would be indirect and could not take proper advantage of the separation from nickel caused abandonment of the method as a practical method for the determination of cobalt.

A study was made of the cobaltous tetrapyridine thiocyanate precipitate announced by Spacu and Dick (1). It was thought possible to oxidize the thiocyanate with iodate as in the copper determination (2).

The neutral solution containing cobalt and ammonium thiocyanate was heated to boiling and pyridine added. When cool the solution was filtered through a crucible with a fritted glass bottom. It was found that 1 ml. of water was sufficient to wash the precipitate. The precipitate was transferred to an iodine flask and titrated with iodate in a strongly acid solution. Analyses of a cobalt salt by this method gave slightly low results.

A volumetric determination of potassium was reported by Bulli and Fernandes (3). This involved the oxidation of the triple nitrite of potassium, cobalt, and lead by an excess of potassium permanganate and titration of the excess permanganate by oxalate.

It was thought possible that this method might be adapted to the determination of cobalt. Saturated solutions of lead nitrate and sodium and potassium nitrites were added to a cobalt salt. The mixtures were agitated and allowed to stand. The precipitate was washed with water and permanganate added. The suspension was stirred thoroughly while being heated to 80°C. Sulfuric acid and oxalate were added, and the solution heated again and titrated.

Although results for the potassium determination are high when small amounts are present (4), the cobalt determination yielded approximately theoretical results when at least two and one-half times the theoretical quantities of precipitating reagents were present.

Somewhat high results were obtained, undoubtedly due to the fact that the precipitation is carried out in concentrated solution.

Since this determination of cobalt could not be made directly on a solution containing both nickel and cobalt, it was thought possible to make a preliminary separation with alpha-nitroso-beta-naphthol. Such a procedure would necessitate destruction of the naphthol. Perchloric acid was found to accomplish this. An evaporation with hydrochloric acid was found to remove the excess of perchloric acid.

Using this method a nickel-cobalt solution was analyzed for cobalt with satisfactory results. A modification which was equally successful was the use of ferrous solution in place of oxalate for the back-titration of part of the samples.

Attempts were made to determine nickel by quantitative oxidation of the dimethylglyoxime precipitate with various oxidizing agents. Potassium permanganate, iodine, ceric sulfate, and hydrogen peroxide were used unsuccessfully.

In the case of the permanganate the glyoxime was heated with an

excess of the reagent. Roughly proportional amounts of oxidizing agent were required for the glyoxime taken, but the results were not very consistent.

The oxidations with iodine were tried with hydroxylamine as a preliminary to use of the glyoxime. This reagent yielded results similar to those obtained with permanganate. Sodium carbonate was added and the solutions heated. In other cases chloroform was used in the titration without apparent increase in oxidation.

Boiling of the nickel precipitate with an excess of ceric sulfate was also tried. Iodine monochloride and silver nitrate were tried as catalysts for this oxidation without much apparent effect. The lack of precision evident in the results caused abandonment of this reagent.

According to Wurster (5) heating of hydroxylamine with hydrogen peroxide at 40°C. gives a quantitative oxidation to nitric acid. This oxidation was attempted for periods up to 20 hours. Approximately 85 per cent of the theoretical was the maximum oxidation obtained.

#### SUMMARY

1. Small amounts of cobalt can be determined by precipitation with alpha-nitroso-beta-naphthol followed by oxidation and titration of the excess naphthol in alkaline potassium permanganate.
2. Cobalt can be determined by titration of cobaltous tetrapyridine thiocyanate with potassium iodate.
3. Cobalt can be titrated by oxidation of potassium cobaltous lead nitrite with potassium permanganate.
4. Oxidation of nickel dimethylglyoxime with potassium permanganate, iodine, ceric sulfate, or hydrogen peroxide is not satisfactory for the determination of nickel.

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# INTERREFLECTION OF LIGHT BETWEEN PARALLEL PLANES<sup>1</sup>

FRANK NOAKES

*From the Department of Electrical Engineering, Iowa State College*

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Using integral equations, H. Buckley (1) studied the interreflections in both finite and infinite cylinders having matt surfaces. The integral equations were solved by a modification of a method suggested by E. T. Whittaker (4). Buckley also analyzed the interreflection between two parallel infinite half-planes of uniform luminosity.

The author, following the work of Parry Moon (3), discusses the theory of interreflections and establishes the integral equations for several cases involving parallel planes including the light court. The approximate solution of one case is obtained by using Whittaker's method; the analytical results are then compared with the experimental results of Meacock and Lambert (2).

## GENERAL THEORY

The luminosity at any point ( $p_1$ ), regardless of the configuration of the boundary surface, is given by

$$L(p_1) = L_0(p_1) + \varrho(p_1) \int_s L(p) \cdot K(p; p_1) \cdot d\sigma$$

where

$p$  = variable point on the boundary surface

$S$  = integration over the surface visible from ( $p_1$ )

$L_0$  = self-luminosity of the surface at the point ( $p_1$ )

$K$  = the illumination at the point ( $p_1$ ) caused by unit luminosity of a unit area at the point ( $p$ )

$d\sigma$  = incremental area of boundary surface at point ( $p$ )

$\varrho$  = reflection factor

The illumination at any point ( $p_1$ ) is given by

$$E(p_1) = E_0(p_1) + \int_s \varrho(p) \cdot E(p) \cdot K(p; p_1) \cdot d\sigma$$

where

$E_0$  = direct illumination and all other quantities are as previously defined.

These two equations are applicable to all problems involving interreflections.

The integral equations for the following specific cases are obtained from this basic theory.

<sup>1</sup>Original thesis submitted July 12, 1940. Doctoral thesis number 573.

- (a) Two parallel infinite half-planes illuminated by a uniform diffuse plane source across one end.

Here

$$E(x) = \frac{L}{2} \left[ 1 - \frac{x}{\sqrt{1+x^2}} \right] + \frac{\varrho}{2} \int_0^\infty \frac{E(\xi)}{[1 + (\xi-x)^2]^{3/2}} d\xi$$

where

$\varrho$  = reflection factor of the matt surfaces of the planes

$x$  = distance, expressed in terms of the separation distance of the planes, from the source to the point at which the illumination is desired.

$\xi$  = distance from the source to the point from which the incremental flux, due to interreflection, is emitted. It is expressed in terms of the separation distance of the planes and is measured along the opposite plane from that on which  $x$  is measured.

- (b) Two parallel infinite planes illuminated by a luminous rod parallel to the planes and equidistant from them.

In this case

$$E(x_{1/2}) = \frac{L \cdot \delta}{4[x^2 + 1/4]} + \frac{\varrho}{2} \int_{-\infty}^{\infty} \frac{E(\xi, 1/2)}{[1 + (\xi-x)^2]^{3/2}} d\xi$$

where

$\delta$  = diameter of the luminous rod and all other quantities are as previously defined.

- (c) Two parallel infinite planes illuminated by a point source half-way between the planes.

The illumination,

$$E(x, 1/2) = \frac{I}{2[x^2 + 1/4]^{3/2}} + 2\varrho \int_0^{\infty} \frac{E(\xi, 1/2) \cdot \xi \cdot (1+x^2+\xi^2)}{[(1+x^2+\xi^2)^2 - 4\xi^2 x^2]^{3/2}} d\xi$$

where

$I$  = intensity of the source (independent of direction) and all other quantities are as previously defined.

In addition to the above equation, for cases b and c, the integral equations for any position of the source in between the planes are established. The analytical approach to the problem of the light court is also outlined.

The solution of a, for the parallel infinite half planes, is given in terms of unit luminosity of the source.

By an adaptation of Whittaker's method the following approximations are made

$$\frac{1}{2} \left[ 1 - \frac{x}{1+x^2} \right] \cong \frac{1}{2} \begin{bmatrix} -0.2504x & -1.3780x \\ 0.0775e & +0.9225e \end{bmatrix}$$

and

$$\frac{1}{[1+x^2]^{3/2}} \begin{bmatrix} -1.4720x & -5.9955x \\ 1.5500e & -0.5500e \end{bmatrix}$$

These two approximations are used in obtaining an approximation for the resolvent kernel and hence the approximate solution of the integral equation.

The approximate solutions in terms of unit luminosity are:

for  $\varrho = 0.2$

$$\Phi(x) = \frac{1}{2} \left[ 1 - \frac{x}{\sqrt{1+x^2}} \right] \begin{bmatrix} -6.4903x & -1.3111x \\ +0.0055e & +1.0913e \\ -0.2504x & -1.3780x \\ +0.0091e & -1.0782e \end{bmatrix}$$

for  $\varrho = 0.4$

$$\Phi(x) = \frac{1}{2} \left[ 1 - \frac{x}{\sqrt{1+x^2}} \right] \begin{bmatrix} -6.1013x & -1.1347x \\ +0.0103e & +0.6435e \\ -0.2504x & -1.3780x \\ +0.0224e & -0.6131e \end{bmatrix}$$

for  $\varrho = 0.6$

$$\Phi(x) = \frac{1}{2} \left[ 1 - \frac{x}{\sqrt{1+x^2}} \right] \begin{bmatrix} -6.1519x & -0.9332x \\ +0.0154e & +0.5123e \\ -0.2504x & -1.3780x \\ +0.0449e & -0.0461e \end{bmatrix}$$

for  $\varrho = 0.8$

$$\Phi(x) = \frac{1}{2} \left[ 1 - \frac{x}{\sqrt{1+x^2}} \right] \begin{bmatrix} -6.2006x & -0.6863x \\ +0.0180e & +0.4371e \\ -0.2504x & -1.3780x \\ +0.0963e & -0.3630e \end{bmatrix}$$

The analytical results are compared with experimental results of Meacock and Lambert; the agreement is very satisfactory.

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# LATERAL ORGANOALKALI COMPOUNDS<sup>1</sup>

HENRY ALEXANDER PACEVITZ

*From the Department of Chemistry, Iowa State College*

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Lateral organoalkali compounds may be defined as those organometallic compounds in which the alkali metal is attached to a carbon chain which is in turn attached to an aromatic nucleus.

Since the most widely studied organoalkali compounds are of this lateral classification, an investigation was undertaken to find methods of preparing some of the more important compounds of this class by simple, inexpensive procedures and to study some of their more interesting properties. Most of the investigation was concerned with compounds of the benzylalkali type because of their potential importance in synthesis.

Benzylsodium was found to be preparable in 77 per cent yields by reacting chlorobenzene with sodium in toluene at 35° and then refluxing the primary reaction mixture for 3 hours. In the primary reaction phenylsodium is formed to an extent of 87 per cent. This yield is superior to that obtained by the method of Ziegler<sup>2</sup> wherein benzene is used as the solvent.

Following a similar procedure, 3,5-dimethylbenzylsodium and β-naphthylmethylsodium were formed to an extent of 20 and 4.3 per cent, respectively.

The preparation of organopotassium compounds by interacting a halide with potassium in ordinary organic solvents usually results in a Wurtz-Fittig reaction. However, we have found that benzylpotassium and 3,5-dimethylbenzylpotassium may be prepared in excellent yields by a technique somewhat similar to the one used in the preparation of benzylsodium. This reaction could never be stopped at an intermediate phenylpotassium stage when toluene was used as the solvent, but benzylpotassium was formed in yields as high as 82 per cent as determined by carbonation reactions. Most probably, phenylpotassium was formed as an intermediate, but immediately reacted with the toluene to form benzylpotassium which is less reactive than phenylpotassium toward chlorobenzene thus interrupting the Wurtz-Fittig reaction. This same interruption occurred when mesitylene was used as the solvent. The yield of 3,5-dimethylbenzylpotassium was 79 per cent as determined by carbonation to form the corresponding acid. For lack of a more exact term, the use of chlorobenzene and potassium in reactions of this type is called metalation by the "chlorobenzene-potassium" system.

Bachmann and Clarke<sup>3</sup> observed that when di-p-tolylmercury and sodium were allowed to react in benzene solution over a period of thirty

<sup>1</sup>Original thesis submitted December 19, 1940. Doctoral thesis number 601.

<sup>2</sup>Ziegler, *Angew. Chem.*, **49**, 457 (1936).

<sup>3</sup>Bachmann and Clarke, *J. Am. Chem. Soc.*, **49**, 2089 (1927).

days, benzoic acid (34 per cent), *p*-toluic acid (30 per cent), and phenylacetic acid (0.36 per cent) were formed subsequent to carbonation of the reaction mixture. The occurrence of phenylacetic acid was attributed to the migration of the sodium atom in the *p*-tolylsodium. However, because of the large amount of benzoic acid which was isolated, there existed the possibility of the metalation of the toluene formed in the reaction by the phenylsodium to give benzylsodium.

We have performed several experiments which show that *p*-tolylsodium, prepared in an inert solvent such as ligroin, undergoes a complete rearrangement to benzylsodium when the reaction mixture is refluxed for several hours. *m*-Tolylsodium and *o*-tolylsodium also undergo a similar rearrangement although to a lesser extent. *p*-Tolylsodium prepared from *p*-chlorotoluene and sodium in toluene gave a 62 per cent yield of phenylacetic acid subsequent to refluxing and carbonation. *p*-Tolylpotassium was found to rearrange and give a 16.5 per cent yield of phenylacetic acid. *p*-Tollylithium could not be induced to rearrange by the method mentioned above. It may be mentioned here that the irregularity of lithium was also observed in the metalation of toluene by *n*-butyllithium inasmuch as only a 0.22 per cent yield of phenylacetic acid was obtained subsequent to carbonation.

In view of the existing uncertainty of the mechanism concerned in the formation of substituted malonic acids when alkyl and lateral aryl alkali compounds are carbonated, an attempt was made to clear up this problem. It was found that the most important unreported condition which controlled the extent of malonic acid formation was the rate at which the organoalkali compound was carbonated. The solvent used and the length of stirring of the organoalkali prior to carbonation were found to be of minor importance. The results of the studies on the effect of the rate of carbonation on the product obtained are given in Table 1. The rapid carbonations were accomplished by pouring the organoalkali compound on crushed solid carbon dioxide over a period of 10 seconds. The

TABLE 1

Organooalkali Compound	Solvent	Monocarboxylic Acid		Substituted Malonic Acid	
		Rapid	Slow	Rapid	Slow
Benzylsodium	Toluene .....	72.9	17.6	0	42.2
Benzylsodium (from dibenzylmercury)	Toluene .....	61	8.5	1	22.8
Benzylpotassium	Toluene .....	82	55	0	23
3,4-Dimethylbenzyl-potassium	Mesitylene .....	79	30	0	15
<i>n</i> -Amylsodium *	Ligroin .....	38.5	15.2	1	16.9
<i>n</i> -Amylsodium	Low boiling petroleum ether .....	51.5	17.3	0	31.4
<i>n</i> -Amylsodium*	Ligroin .....	30		9.7	

\* The rapid carbonation in this experiment was carried out by the use of a very rapid stream of carbon dioxide gas instead of crushed solid carbon dioxide.

slow carbonations were performed by admitting dried gaseous carbon dioxide over the surface of the stirred suspension over a period of 4 to 5 hours, after which a negative color test<sup>4</sup> was obtained.

Attempts to prepare benzylcalcium chloride, *p*-tolylcalcium chloride, and *p*-tolylcalcium bromide by methods similar to those used in preparing the corresponding organoalkali compounds all gave negative results.

Several interconversion reactions were studied using *n*-butyllithium and various substituted aryl bromides. The amount of corresponding acid isolated on interconversion of each of the following halides with *n*-butyllithium is given in parentheses: 2-bromodibenzothiophene (17 per cent); 2,4,6-trimethylbromobenzene (40 per cent); 2,4,5-trimethylbromobenzene (63 per cent); *p*-bromo-*t*-butylbenzene (86 per cent).

The first interconversion of an organosodium compound and an organic halide was accomplished when *n*-butylsodium was found to undergo an interconversion with  $\alpha$ -bromonaphthalene. Subsequent to carbonation of the reaction mixture, a 25.6 per cent yield of  $\alpha$ -naphthoic acid was obtained.

With respect to the results obtained in the migration studies involving the shift of metal from the nucleus to the side chain on heating, it was thought that the allylic rearrangement of benzylmagnesium chloride would be hindered by such treatment. Contrary to this prediction, it was found that the allylic rearrangement was enhanced by heating, occurring to an extent of 10.5 per cent at 15° and 18 per cent at 90°.

<sup>4</sup> Gilman and Schultz, *J. Am. Chem. Soc.*, 47, 2002 (1925).

# PROPERTIES OF COMPOUNDS PRODUCED BY THE ACTION OF ACETOBACTER SUBOXYDANS UPON i-INOSITOL<sup>1</sup>

WALTER H. PITCHER

From the Department of Chemistry, Iowa State College

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i-Inositol is a hexa-hydroxy cyclohexane compound occurring widely in nature mainly as the methylether and phosphoric acid ester derivatives. Previous investigations have shown that under appropriate conditions *Acetobacter suboxydans* is able to oxidize i-inositol to form a cyclic polyhydric ketose which preliminary studies indicated to be a diketo-i-inositol. Since the exact chemical nature of the product was not known, it was referred to as "ketose," a term used throughout this thesis.

Not only was it the object of this investigation to study the chemical properties of the fermentation product, but also to determine whether or not the Bios I activity of i-inositol had been affected by the limited oxidation involved.

The culture of *Acetobacter suboxydans* used in the experiments for this thesis was obtained from the American Type Culture Collection and is listed as No. 621. The stock cultures were carried on glycerol-yeast extract agar slants, but subculture and fermentations were performed using liquid medium containing 3 per cent i-inositol, 0.5 per cent yeast extract (Difco), and 0.1 per cent sorbitol. "Ketose" was separated from the culture medium by treating the medium with basic lead acetate, filtering, removing the excess lead as the sulfide, and then distilling the filtrate so obtained *in vacuo* to crystallization. Purification consisted in repeated recrystallizations from water and 60-70 per cent ethyl alcohol.

The course of the conversion of i-inositol into the reducing fermentation product was followed by the use of the Shaffer-Somogyi semi-micro method as modified by Guymon. The correct heating time and reducing curve were determined by preliminary experiments with a standard solution of "ketose."

Previous investigations have shown that the optimum pH range of the unbuffered medium was 5.1 to 6.8. A fermentation series was run containing phosphate buffered medium at pH 6.4, 6.0, and 5.8. The results showed that there was a definite relationship between the formation of reducing compounds and the pH of the fermenting medium; the higher the buffered pH, the less reducing material formed.

Further evidence that "ketose" is predominantly a diketo compound was obtained from quantitative hydrogenation and oxidation experiments. The volume of hydrogen necessary to reduce a given weight of "ketose" to i-inositol in the presence of platinum oxide catalyst corresponded to

<sup>1</sup> Original thesis submitted March 17, 1941. Doctoral thesis number 604.

about 78 per cent diketo-i-inositol. Oxidation equivalent values were based upon the complete oxidation of the fermentation product by iodic acid. The excess iodic acid was measured by adding potassium iodide and titrating the iodine liberated with standard sodium thiosulfate solution. The oxidation values thus obtained, agreed with those calculated for a diketo-compound.

The methylation of "ketose" was accomplished by refluxing with hot alkaline dimethyl-sulfate for 24 hours. The methylated compound was obtained as a syrup which gave a positive analysis for methoxyl content. The preparation of a fully methylated derivative should permit an oxidative rupture of the cyclic ring adjacent to the carbonyl groups, and identification of the fragments should give significant information as to the structure of the "ketose."

In view of the fact that i-inositol has been identified as an indispensable growth factor for various yeast species; one would expect "ketose" to exhibit similar properties. If i-inositol could be biologically oxidized to keto compounds, which might exist in reversible oxidation-reduction systems, some light might be thrown upon its role as Bios I. Consequently, "ketose" was tested in twenty-nine combinations with nine known yeast stimulants upon each of three strains of *Saccharomyces cerevisiae*. Growth was measured by means of a K.W.S.Z. photometer which in turn was calibrated by microscopic count. The basal synthetic medium used for all the subsequent experimental fermentations contained per 100 ml., 0.188 grams of ammonium chloride, 0.100 grams dipotassium phosphate, 0.100 grams of calcium chloride, 0.100 grams magnesium sulfate, and 5 grams of sucrose. Yeast growth was measured at 0, 12, 24, 48, 72, 96, and 120-hour intervals. Criteria for comparisons included "final photometer reading" and "hours to average photometer reading."

Results showed that in all combinations "ketose" not only played the same role as Bios I in yeast growth, but in the majority of cases the stimulating effect due to "ketose" was slightly greater than that due to i-inositol. Since quebrachitol, the monomethyl ether of i-inositol, does not stimulate yeast growth, it appeared that some sort of oxidation-reduction reaction was necessary for Bios I activity.

The possibility that the activity of "ketose" was due to adsorbed yeast extract from the *Acetobacter* fermentation was dealt with by purifying "ketose" through the phenylhydrazone derivative and subsequent recovery of the original keto compound. This "purified ketose" gave the same stimulating effect as the original "ketose," thus proving the absence of adsorbed yeast extract.

Phytin (a hexaphosphoric acid derivative of i-inositol) occurs widely in plants. Results of tests upon the effect of phytin and the tetraphosphoric acid ester of i-inositol upon yeast growth showed that both possessed Bios I activity. Hence the yeast cell must possess phosphatase enzymes. This is in contrast to *Acetobacter suboxydans* which is unable to ferment ether phytin or the tetraphosphoric acid ester of i-inositol.

# PRODUCTION OF CHEMICALS BY THE FERMENTATION OF THE ACID HYDROLYZATE OF OAT HULLS<sup>1</sup>

MORTON M. RAYMAN

From the Department of Chemistry, Iowa State College

Received May 22, 1941

The accumulation of agricultural residues by certain industries has created a serious disposal problem. The economical utilization of such residues is not only of importance to the industry, but the resultant accumulation represents a loss to the producer of the raw material. For several years the fermentative utilization of these materials has been the subject of investigations in these laboratories. Most of this research has been based upon oat hulls which represent a typical residue produced in Iowa. Oat hulls, the waste product of the industrial processors of oats, are produced at the rate of 400 tons daily at the factories in the United States. Of the possible methods of disposing of this trade waste, the approach by fermentation of the pentose sugars in the oat hulls was chosen for these experiments. The pentose fraction, largely xylose, constitutes approximately one-third of the weight of the oat hulls, and is set free in solution when the hulls are given a mild hydrolytic treatment with hot dilute mineral acids.

Oat hulls were treated with dilute mineral acids at 20 pounds steam pressure for 60 minutes. A liquid : solid ratio of 6 : 1 (i. e., six milliliters of acid per gram of hulls) was used; the concentration of acid, the time of hydrolysis, and the temperature employed were found to be optimum. Sulfuric or hydrochloric acids were commonly used at a concentration of 0.08 molar. The crude sugar filtrate was neutralized with calcium carbonate, and sodium hydroxide solution was added to adjust the pH to a value of about 6. The evaporation of this solution *in vacuo* at 50-55° C., gave an oat hull sirup which could be conveniently diluted to the proper concentration for the fermentation experiments. After the nutrients had been added to the sugar solutions, the medium was sterilized in the autoclave preliminary to inoculation. Micro-organisms of potential industrial importance were chosen for the tests. This thesis reports the behavior of two species of bacteria and two of molds selected for the work.

Since corn mash is the most favorable medium for the development of *Clostridium acetobutylicum*, the experimental fermentation media were prepared by mixing oat hull hydrolyzate with corn mash. The media contained, usually, 4 to 5 per cent of total reducing sugars. Normal yields of solvents were obtained, and the ratio of butanol, acetone, and ethanol were essentially 6 : 3 : 1. As much as 50 per cent of the total carbohydrate could be derived from the oat hull sugars without diminish-

<sup>1</sup> Original thesis submitted July 9, 1940. Doctoral thesis number 572.

ing the solvent production. Hydrolyzates prepared with hydrochloric, nitric, sulfuric, or phosphoric acids were fermented equally well by the action of the butyl-acetonic organism on mixtures containing corn mash. Attempts to ferment the sugars of the hydrolyzate in the absence of corn gave little or no solvents. Of special interest was the fact that hydrolyzates prepared with phosphoric acid gave abnormal fermentations in which the activity of the cultures soon ceased and the production of butyric and acetic acids was markedly high. Control fermentations in which pure xylose was supplemented by corn gluten or soybean meal gave normal yields of solvents. Similar results were obtained on hydrolyzates of purified xylan isolated from oat hulls by an alkaline extraction.

The main products of *Aerobacter aerogenes* fermentations of xylose are ethanol, carbon dioxide, and 2,3-butylene glycol. The organism, obtained from the American Type Culture Collection, was maintained on glycerol-yeast extract agar for stock cultures. The medium employed in the experimental fermentations contained the following constituents, the concentration being expressed in grams per liter of medium:

NH <sub>4</sub> Cl .....	3.0
K <sub>2</sub> HPO <sub>4</sub> · 3H <sub>2</sub> O .....	1.75
MgSO <sub>4</sub> · 7H <sub>2</sub> O .....	1.75
CaCl <sub>2</sub> .....	0.1
Xylose .....	50.0

The pH of the medium was adjusted to a value of 6.25 by the addition of a solution of sulfuric acid. The medium was sterilized at 10 pounds steam pressure for 30 minutes. After inoculation, samples were removed daily for sugar analysis. Requisite amounts of sterile molar sodium carbonate solution were added daily to readjust the pH to 6.25. The medium containing pure xylose showed typical fermentative activity and gave, on analysis, yields of 2,3-butylene glycol similar to values reported in the literature. Practically all the sugar in the medium was consumed in the fermentation. However, parallel experiments in which the medium contained oat hull sirup, equivalent in concentration to the xylose medium, showed no signs of growth or of fermentation.

Of the molds which utilize xylose *Aspergillus flavus* offered some promise. The main product, kojic acid, had been reported by several investigators. The medium employed had the following composition, the values being expressed in grams per liter of medium:

NH <sub>4</sub> NO <sub>3</sub> .....	1.00
KH <sub>2</sub> PO <sub>4</sub> .....	0.625
MgSO <sub>4</sub> · 7H <sub>2</sub> O .....	0.500
Xylose (equivalent to that in oat hull sirup) .....	128.0

The culture used was obtained from Dr. Charles Thom and was maintained on glycerol-yeast extract agar. The kojic acid analyses of the sugar media were made by two methods. When a pure xylose medium was to be examined, the kojic acid was precipitated as the copper salt and weighed. With an oat hull sugar medium this method was not suitable

since other substances in the oat hull sirup would precipitate along with the kojic acid when cupric acetate was added as a precipitant. Hence, under the above conditions, the cultures were extracted continuously with ether. The kojic acid in the extract was recrystallized from acetone and weighed. Yields of kojic acid were substantially better in the case of the medium containing pure xylose than in the case where oat hull sirup had been used as a substrate.

Since *Penicillium chrysogenum* is known to convert glucose to gluconic acid in a suitable medium, it was to be expected that xylose might be oxidized to xylonic acid in the presence of the mold. The organism was obtained from Dr. Charles Thom, and stock cultures were maintained on glycerol-yeast extract agar. The experimental medium possessed the following composition; the concentrations are expressed in terms of grams per liter.

$\text{NaNO}_3$	.....	3.0
$\text{KH}_2\text{PO}_4$	.....	0.300
$\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$	.....	0.250
$\text{FeCl}_3$	.....	0.100
$\text{H}_3\text{BO}_3$	.....	0.250
$\text{CaCO}_3$	.....	40.0
Xylose (pure or its equivalent from oat hull sirup)	....	200.0

Immediately after inoculation of the media, vigorous aeration was begun and periodic analysis for residual sugars was made during the fermentation. After fifteen days incubation at 30° C., the cultures were filtered hot and the soluble calcium determined in the medium was used as a measure of calcium xylonate formation. Xylonic acid was isolated and characterized as its brucine derivative, m.p. 170-2° C. Based on the sugar consumed, the apparent yield of xylonic acid was 25.8 per cent for the xylose medium; for the parallel fermentation with oat hull hydrolyzate, it was 52.9 per cent. The above difference may be attributed largely to greater assimilation by the mold in the oat hull medium and more disimilation in the xylose medium.

A discussion of the possible factors present in oat hulls which inhibit the fermentation is presented. Among the compounds considered are the furanoid condensation products of pentose-lignin combinations, lignins, saponins, tannins, and certain complex nitrogenous compounds. Suggestions are made on methods of removing some of these inhibiting factors.

# FLUORESCENCE ASSOCIATED WITH PROTEINS<sup>1</sup>

WENDELL REEDER

*From the Department of Chemistry, Iowa State College*

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Although fluorescence of biological materials has been observed by numerous investigators, few detailed studies have been made to determine the specific constituents present in these materials which are responsible for the emission of light. The color of protein fluorescence has been recorded in the literature, but no detailed study of the effects of protein treatment on fluorescence has been made. The present investigation was undertaken to determine whether or not the fluorescence of proteins could be used in protein chemistry. More specifically, the objectives were: first, to determine whether or not the fluorescence of proteins is due to occluded or adsorbed material; second, to determine the effect of protein hydrolysis by various methods on the fluorescence of the proteins; and third, to study the products of protein hydrolysis and determine which of the protein constituents are fluorescent.

The relative fluorescence intensities of the proteins and their hydrolyzates were determined by measuring the dilution required to reduce the fluorescence of a given amount of protein or protein hydrolyzate to the same intensity as the fluorescence of a diluted standard solution of quinine bisulfate.

The proteins prepared and studied in this investigation were casein, wheat gluten, gliadin, glutenin, blood fibrin, gelatin, ovalbumin, and zein. Hair and wool were also compared to the proteins.

When examined in ultra-violet light of wavelengths 3,100-4,100 Å, the proteins give a uniform bluish-white fluorescence in the solid state and a somewhat more green fluorescence in solutions. The fluorescence of these proteins is more green in basic solution than in acid, but the color change is not sharp.

The fluorescence of proteins is destroyed by oxidation with strong nitric acid or by ashing. The small amount of protein ash is not fluorescent in the solid state or in acid, basic, or neutral solution.

Various methods of extraction with organic solvents were used in attempts to remove the fluorescent material from the proteins. Organic solvents do not extract the fluorescent material from the solid protein or from the protein hydrolyzates in acid or basic solution. Likewise, dialysis experiments failed to remove the fluorescent material from protein solutions, but after hydrolysis with strong acid the fluorescent material is readily removed from proteins by dialysis.

The effect of hydrolysis of proteins by means of acids, bases, and

<sup>1</sup> Original thesis submitted July 16, 1940. Doctoral thesis number 589.

proteolytic enzymes on the fluorescence was determined. It was found that hydrolysis by proteolytic enzymes or alkali produced only a slight increase in the amount of fluorescence. However, hydrolysis with hydrochloric acid, sulfuric acid, perchloric acid or phosphoric acid produced large increases in the fluorescence of those proteins containing tryptophane and only slightly increased fluorescence in those proteins which are deficient in this amino acid. The color of the fluorescence produced during acid hydrolysis is blue-green.

Crude commercial proteins produce the same amount of fluorescence, during acid hydrolysis, as these same proteins prepared in a purified state. The presence or absence of air during acid hydrolysis does not affect the amount or color of fluorescence of the protein hydrolyzates.

Nineteen amino acids were examined in ultra-violet light for fluorescence. These amino acids were not noticeably fluorescent in the solid state or in acid, basic, or neutral solution.

Amino acid additions to proteins during hydrolysis revealed that tryptophane was the only amino acid which increased the amount of fluorescence during acid hydrolysis. Tryptophane did not affect the amount of fluorescence produced during hydrolysis of proteins with alkali.

When proteins such as zein and gelatin, which are deficient in tryptophane, are hydrolyzed in the presence of this amino acid, the amount of fluorescence is greatly increased. However, when tryptophane is added to proteins containing this amino acid, the fluorescence is only slightly increased during acid hydrolysis.

The addition of the vitamins which are capable of producing blue-fluorescent compounds did not affect the amount or color of fluorescence during acid hydrolysis.

Boiling acetic acid with proteins greatly increased the amount of fluorescence. However, the fluorescent color produced is much more blue than that of protein solutions or hydrolyzates. Boiling acetic acid with several of the amino acids caused a blue fluorescence to appear in the solutions. This is in contrast to the action of strong acids on these same amino acids. Strong acids do not produce fluorescence of the amino acids. This would indicate that the fluorescence increase with acetic acid and protein is due to a different substance than is produced during acid hydrolysis with strong acids.

The production of artificial melanins by the action of glucose on tryptophane or tyrosine in acid solution also gives rise to a blue-green fluorescence. Tyrosine and tryptophane were the only amino acids studied which gave blue-green fluorescence with glucose. The melanin produced by the action of the tyrosinase of potato juice on pure tyrosine solutions was not fluorescent.

A method of concentrating the fluorescent material from protein hydrolyzates was developed. It involved adsorption of the fluorescent material from acid solutions with English fuller's earth and subsequent elution from this adsorbant with alcohol-ammonium hydroxide solutions.

Some of the properties of this fluorescent concentrate are given. From its solubility properties, the fluorescent material was found to be different from the blue-fluorescent alkaloid harman produced by the mild oxidation of the acetaldehyde-tryptophane complex.

The fluorescent spectra of lactoflavin, thiochrome, quinine bisulfate, and the fluorescent material from protein hydrolyzates were compared. These compounds all give broad bands of fluorescence light. The fluorescent spectrum of the material concentrated from casein hydrolyzate is in the blue-green region of the spectrum and is very similar to the fluorescent spectrum of quinine bisulfate. However, these two compounds are not identical since they can be easily separated by ether extraction of a basic solution containing both compounds. The fluorescent spectrum of the material concentrated from casein hydrolyzate is in the blue-green region having wavelengths 4,100-5,300 Å. The fluorescence is excited by light of wavelengths 3,400-3,600 Å.

# THE DIELECTRIC CONSTANT OF GASES AT ULTRA-HIGH FREQUENCIES<sup>1</sup>

ALDEN HOOVER RYAN

*From the Department of Physics, Iowa State College*

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With the growing use of the ultra-high frequencies in applied physics, redetermination of the dielectric constants of gases appears desirable at these frequencies. The purpose of the present investigation was twofold: (1) to determine whether the heterodyne beat method could be used successfully at frequencies up to 56 megacycles per second; (2) to determine the dielectric constant of three typical gases (nitrogen, ammonia, and carbon dioxide) at a frequency around 56 megacycles.

The principal difficulties of the heterodyne beat method are to maintain the necessary frequency stability of the two oscillators and to prevent their synchronization at low beat frequencies. These difficulties were overcome by using great care in the construction and operation of the apparatus. The fixed oscillator was a temperature-regulated crystal controlled oscillator. The variable oscillator used a tube (the RCA 955) especially designed for ultra-high frequencies in an electron coupled circuit. It was isolated from the detector by a buffer stage. The temperature of both oscillators was kept as nearly constant as possible. To reduce synchronization, both oscillators were shielded very carefully, and were coupled very weakly, by radiation to the detector.

After detection, the beat note was amplified by a two-stage audio-amplifier and was then measured by comparing it with a calibrated audio-frequency, using a cathode-ray oscilloscope to indicate zero beat.

The test condenser was made of two concentric cylinders, supported on Pyrex tubing in such a way that the fixed capacity (not affected by admitting the gas) was minimized. A remote control system was used to tune the oscillators and admit gas to the test condenser. The condenser and a one-liter flask, used as a reservoir of the gas, were placed in a metal box for electrical shielding and to improve the temperature control of the condenser.

Readings were made over a range of temperature from 22.36° C. to 47.33° C. The temperature at any point was maintained within 0.05° C. A series of readings was made on nitrogen, ammonia, and carbon dioxide at a frequency of 56.72 megacycles. A second series of readings was taken on nitrogen and carbon dioxide at a frequency of 28.36 megacycles. The difference between atmospheric pressure and the pressure in the condenser after admitting the gas was measured on a mercury manometer, which could be read by means of a lens arrangement to one-tenth of a millimeter. Atmospheric pressure was obtained from a standard mercury barometer.

<sup>1</sup>Original thesis submitted July 18, 1939. Doctoral thesis number 541.

The consistency of the results obtained showed that the heterodyne method can be used successfully at ultra-high frequencies. The random errors (due primarily to oscillator instability) amounted to less than one-half of one per cent, for the data on nitrogen, and less than one-third of one per cent, for the data on ammonia and carbon dioxide. Difficulties of calibration would raise the probable error in the absolute values of (dielectric constant - 1) to about 1 per cent.

There appears to be no change in the dielectric constant of these three gases with frequency up to 56 megacycles. The values obtained for the dielectric constant of these gases at 56 megacycles agreed with the results of previous observations as well as those results agree among themselves. Furthermore, the values of the dielectric constant of nitrogen and carbon dioxide measured at 56.72 megacycles were the same, within experimental error, as when measured at 28.36 megacycles.

An anomalous variation of the dielectric constant of carbon dioxide with the temperature was observed. The value of (dielectric constant - 1) increased with temperature from the value it had at 22° C. to a maximum at about 39° C., and then decreased again toward the value it had at the lower temperature. The same effect was observed both at 56 megacycles and at 28 megacycles. The temperature at which the maximum occurred was almost the same for both frequencies.

This effect was discussed and a comparison made with the data of other observers (1). It was concluded that while the data of some observers (4) showed a similar effect, that of others (5) showed no such effect, or a slight effect in the opposite direction.

Possible explanations for the effect are discussed on the basis of a change in the structure of the CO<sub>2</sub> molecule. The bearing on these explanations of measurements made by other observers on the specific heat of carbon dioxide (3), its viscosity (2), and its electric moment (6) are mentioned.

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# A CHEMICAL INVESTIGATION OF AMERICAN VERATRUM<sup>1</sup>

EDWIN J. SEIFERLE

From the Department of Chemistry, Iowa State College

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The present study of *Veratrum viride* Ait., a liliaceous plant indigenous to North America, whose rhizomes and roots are used as a crude drug and as a stomach poison against insects such as the currant worm, was undertaken to improve the procedure for determination of the total alkaloid content of the plant, to separate the crude, physiologically active alkaloid mixture extracted from the plant into its constituents, and to test them for toxicity to insects.

An extensive study of the chemical method for assay of *V. viride* was made by Viehoefer and Clevenger,<sup>2</sup> and a fairly satisfactory procedure was developed by them. In the present work several modifications of this method were made which resulted in increased ease of manipulation and accuracy of results.

The most recent contribution to the chemical study of *V. viride* was made by Wright<sup>3</sup> in 1879. He isolated three alkaloids from the plant, jervine, pseudojervine, and rubijervine. Jervine had previously been found in *V. album*, the European species, by Simon<sup>4</sup> in 1837, and the latter pair had been discovered by Wright and Luff<sup>5</sup> in 1879 as components of *V. album*. Subsequent work by Salzberger<sup>6</sup> has resulted in the separation from *V. album* of another alkaloid, protoveratrine, which was highly toxic to vertebrates.<sup>7</sup> In 1937-1938 Poethke<sup>8</sup> found all these alkaloids in *V. album* and further isolated a new, toxic alkaloid, germerine.

In this study the crude alkaloid mixture from *V. viride* was investigated with a view to separation into its components. Five alkaloids were isolated in pure form and identified. These were jervine, pseudojervine, rubijervine, protoveratridine, and germine. Jervine and pseudojervine were found to be the predominant alkaloids separated from *V. viride*, jervine being obtained as about 17 per cent of the total crude alkaloid fraction and pseudojervine as about 3.3 per cent of the crude; rubijervine was obtained in small amounts. Since these three alkaloids had previously been isolated from *V. viride*, their presence in the plant was thus confirmed. Protoveratridine and germine were obtained from *V. viride* for the first time, the former in small amounts and the latter in somewhat

<sup>1</sup>Original thesis submitted July 15, 1940. Doctoral thesis number 577.

<sup>2</sup>Viehoefer and Clevenger, *J. Am. Pharm. Assoc.*, 11:166 (1922).

<sup>3</sup>Wright, *J. Chem. Soc.*, 35: 421 (1879).

<sup>4</sup>Simon, *Ann. Phys. Chem.*, [2] 41: 569 (1837).

<sup>5</sup>Wright and Luff, *J. Chem. Soc.*, 35: 405 (1879).

<sup>6</sup>Salzberger, *Arch. Pharm.*, 228: 462 (1890).

<sup>7</sup>Eden, *Arch. Exptl. Path. Pharmakol.*, 29: 440 (1892).

larger quantities. Poethke<sup>8</sup> had previously shown that germine and 1-methylethylacetic acid were produced on hydrolysis of protoveratridine and that protoveratridine and 1-methylethylglycolic acid were produced on partial hydrolysis of germerine.

Neither germerine nor protoveratrine was obtained in these investigations on *V. viride*, but their presence in this plant is not thereby excluded. In fact, the existence in the plant of some such alkaloid as germerine is indicated by the very fact of the isolation of germine and protoveratridine, and also by the considerable toxicity of the alkaloidal fractions from which these compounds were separated.

Because of the suggested homology between germine and the alkaloid, cevine, the basic hydrolysis product of cevadine and veratridine from commercial veratrine (prepared from sabadilla seeds, *Schoenocaulon officinale* Gray), the reactivities of these two alkaloids to catalytic hydrogenation were studied. Jacobs and Craig, who are studying the structure of cevine,<sup>9</sup> have hydrogenated cevine in the presence of Raney nickel. In this work cevine was hydrogenated with Adams and Shriner's platinum oxide catalyst, but germine could not be successfully hydrogenated with either of these catalysts. It is concluded, therefore, that the two alkaloids are probably not structurally related to the extent suggested.

The toxic properties of many fractions separated during this investigation, including those from which protoveratridine and germine were obtained, as well as of some of the pure alkaloids isolated, were tested by microinjection of their solutions into the American cockroach, *Periplaneta americana* (L.). In this way the progress of the fractionation procedures and the concentration of the toxic components by these procedures were followed biologically. Further, the alkaloids, jervine and pseudojervine, were found to be almost nontoxic to the cockroach and the median lethal dose of germine was determined to be about 0.3 mg. per g. for the American cockroach. These toxicological experiments with pure alkaloids were the first in which pure alkaloids from *Veratrum viride* were tested on insects.

This investigation was financed by the Industrial Science Research Institute of Iowa State College.

<sup>8</sup>Poethke, *Arch. Pharm.*, 275:357, 571 (1937); 276:170 (1938); *Scientia Pharm.*, 9:110 (1938).

<sup>9</sup>Jacobs and Craig, *J. Biol. Chem.*, 119:141 (1937); 120:447 (1937), 124:659 (1938); 125:625 (1938); Craig and Jacobs, *ibid.*, 129:79 (1939); *J. Am. Chem. Soc.*, 61:2252 (1939); *J. Biol. Chem.*, 134:123 (1940).

# EFFECT OF CARBON DIOXIDE AND ACIDS ON SURVIVAL OF MICRO-ORGANISMS<sup>1</sup>

CLIFFORD ALLEN SHILLINGLAW

From the Department of Bacteriology, Iowa State College

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In a study of the survival of intestinal organisms in solutions of edible acids, it was found that the course of death of both *Escherichia coli* and *Eberthella typhosa* was essentially semilogarithmic. Determinations of survivors were made both by plate and tube counts, and the times required for equinormal solutions of the various acids to effect a 99.999 per cent reduction in numbers of *Esch. coli*, (called the killing time) at 30°C., were: citric—57½ hours, acetic—43½ hours, lactic—12 hours, phosphoric—8½ hours, glycolic—6 hours, and tartaric—4¼ hours. In the series of acids tested it was noticed particularly that a hydroxyl group on the α carbon, next to the carboxyl group, seemed to give an acid a high germicidal activity.

Four of the acids were also tested for germicidal activity against *Esch. coli* at 33°F. (5/9°C.), and it was found that the ratio of killing times at 33°F. to the killing time of the same acid at 30°C. were: citric—2.3, tartaric—15.8, phosphoric—5.4, and lactic—3.9.

The addition of 10 per cent sucrose to the acid solutions increased slightly the rate of death of *Esch. coli*. Addition of three volumes of carbon dioxide to the acid solutions resulted in a threefold increase in the germicidal efficiency of citric acid, and an approximate 20 per cent increase in the germicidal efficiency of lactic acid. Three volumes of carbon dioxide in water increased the death rate of *Esch. coli* only very slightly above what it was in water alone.

*E. typhosa* was much more sensitive to the action of acids than was *Esch. coli*; the killing time for *E. typhosa* in lactic acid being 1/6, and in citric acid approximately 1/3, that for *Esch. coli*. Three volumes of CO<sub>2</sub> in water had a marked germicidal action against *E. typhosa*, the rate of death being about three times what it was in uncarbonated water.

The rates of death of *Esch. coli* in commercial soft drinks were found to be essentially what would have been predicted from the acid, sugar, and carbon dioxide contents of the beverages. Size of inoculum used in an acid solution seemed to have no effect on the rate of death of the organism.

Studies were made on the inhibition of yeast growth by benzoic, monochloracetic, and sulfurous acids, and all were found to act as inhibitors. In media adjusted to different hydrogen-ion concentrations, all three compounds caused the growth of yeast to be inhibited at higher

<sup>1</sup> Original thesis submitted June, 1941. Doctoral thesis number 625.

pH values, and the greater the concentration of the inhibiting compounds the higher were the pH values at which inhibition occurred.

Three yeast cultures were used and, when either chloracetic or benzoic acid was present in a medium, all yeast cultures were inhibited at essentially the same pH value. Inhibition was also found to occur at essentially the same pH values, when either monochloracetic or benzoic acids were present, in each of three different media chosen to present a wide variance in amount of nutritive substances for yeast growth.

The inhibiting effect of chloracetic acid was found to be due specifically to the chlorinated molecule, since acetic acid in the same molar concentrations had no effect on the pH value at which inhibition occurred.

Benzoic acid seemed to be much more effective, either from the standpoint of concentration of the compound required to inhibit, or pH value at which inhibition would occur with a given concentration, than was chloracetic acid.

Sulfurous acid in concentrations of 100 to 200 ppm, calculated as SO<sub>2</sub>, seemed to be quite effective for inhibiting yeast growth, and more SO<sub>2</sub> was required to inhibit growth in malt broth than was required in the sugar medium.

A modified Lebedev yeast juice was found to be very slightly active, from the standpoint of CO<sub>2</sub> evolution and O<sub>2</sub> uptake, at pH 5.0, but quite active at pH 5.3 and 5.5. The inhibiting actions of benzoic and monochloracetic acids on the enzymes of this juice were found to be much more manifest at pH 5.3 than at 5.5, indicating that the effect of pH on the ability of such compounds to inhibit yeast growth is due to the fact that the compounds act as enzyme inhibitors only at the more acid reactions.

# SOME METALLIC OXIDES AS PROMOTERS FOR COPPER OXIDE CATALYSTS IN THE HYDROGENATION OF FURFURAL<sup>1</sup>

MEREDITH MARVIN STEWART

From the Department of Chemistry, Iowa State College

Received May 22, 1941

Improved methods have made the hydrogenation of organic compounds a simple and convenient process. The use of high pressure, pioneered by Ipatieff (2), permits hydrogenations to be carried out in the liquid phase. Sabatier (4) and his associates developed many of the catalysts which are in use today. New alloys have made possible the construction of high pressure apparatus which is very safe and convenient.

The hydrogenation of furfural in the liquid phase has been the subject of several investigations. Menzel (3) studied the effect of various pressures and temperatures on this hydrogenation in these laboratories. Adkins (1) and his associates investigated the use of various catalysts in the hydrogenation of furfural and found that several products were obtained. The copper-chromium oxide catalyst developed by Adkins converted furfural into furfuryl alcohol, while a nickel catalyst changed furfural into tetra-hydro-furfuryl alcohol. Menzel found that a catalyst composed of cuprous oxide and calcium oxide produced a smooth and quantitative conversion of furfural to furfuryl alcohol. Though this catalyst was very active and quite selective, it lacked stability and was found to be ineffective in the hydrogenations of ketones due to the conversion of the cuprous oxide to free copper.

## EXPERIMENTAL

The hydrogenation of furfural in the liquid phase was carried out in a large-sized rocking bomb. This vessel was approximately 3 feet long, 3 inches in inside diameter, and had a capacity of 3.85 liters. The bomb was fitted with electrical units capable of raising the temperature to 250° C. The initial hydrogen pressures were approximately 600 pounds. The following catalysts were used in this study:

(A) A series of catalysts containing Cu<sub>2</sub>O co-precipitated with Al<sub>2</sub>O<sub>3</sub>, Cr<sub>2</sub>O<sub>3</sub>, Co(OH)<sub>2</sub>, and Mn(OH)<sub>2</sub> and promoted with CaO.

(B) A series of catalysts containing Cu<sub>2</sub>O promoted by CaO and another metallic oxide. ZnO, SnO, Al<sub>2</sub>O<sub>3</sub>, Cr<sub>2</sub>O<sub>3</sub>, CoO, Mo<sub>2</sub>O<sub>5</sub>, and V<sub>2</sub>O<sub>4</sub> were used as the supplementary promoter.

(C) Catalysts containing Cu and CuO promoted by CaO and V<sub>2</sub>O<sub>4</sub>.

The preparation of the catalysts and promoters is given below.

1. Cu<sub>2</sub>O was prepared by the reduction of an alkaline solution of cupric nitrate by glucose. The precipitate was dried under vacuum at room temperature for 12 hours.

<sup>1</sup>Original thesis submitted March 16, 1940. Doctoral thesis number 552.

2. CaO was prepared by the dehydration of  $\text{Ca}(\text{OH})_2$  at 600-700° C.
  3. The co-precipitated catalysts were prepared by treating an alkaline solution containing cupric nitrate and aluminum, chromium, manganese, or cobalt nitrate with glucose.
  4. CuO, CoO,  $\text{Al}_2\text{O}_3$ ,  $\text{Cr}_2\text{O}_3$ , and SnO were prepared by precipitating the hydroxides and dehydrating them at 230-250° C.
  5.  $\text{V}_2\text{O}_4$  and  $\text{Mo}_2\text{O}_5$  were prepared by the reduction of  $\text{V}_2\text{O}_5$  and  $\text{MoO}_3$ .
  6. Cu was prepared by reducing  $\text{Cu}_2\text{O}$  with hydrogen at 200-220° C.
- Two hundred and fifty ml. of furfural together with the catalyst were placed in the bomb and 600' pounds pressure of hydrogen was added. The rocking mechanism was started, and the bomb was heated until a temperature of 225° C. was reached. The course of the reaction was followed by recording the temperature and pressure at 5-minute intervals until the absorption of hydrogen was completed. All pressures were reduced to 0° C., and from these corrected pressures the activities of the various catalysts were calculated.

#### CONCLUSIONS

1. Co-precipitation of  $\text{Cu}_2\text{O}$  with oxides of an acidic or amphoteric character was found to be an unsatisfactory method for the preparation of hydrogenation catalysts.
2. Catalysts of the type tested in this study required very careful drying.
3. The addition of ZnO,  $\text{Al}_2\text{O}_3$ , CoO, SnO, or  $\text{Mo}_2\text{O}_5$  did not increase the activity of a  $\text{Cu}_2\text{O}-\text{CaO}$  catalyst.
4.  $\text{Cr}_2\text{O}_3$  prepared by the dehydration of  $\text{Cr}(\text{OH})_3$  at 250° C. under reduced pressures was a good promoter for the  $\text{Cu}_2\text{O}-\text{CaO}$  catalyst.
5.  $\text{Cr}_2\text{O}_3$  prepared by the decomposition of  $\text{Cr}(\text{NO}_3)_3$  at 300-350° C. was not an effective promoter for a  $\text{Cu}_2\text{O}-\text{CaO}$  catalyst.
6. A catalyst composed of  $\text{Cu}_2\text{O}-\text{Cr}_2\text{O}_3-\text{CaO}$  mixed by mechanical means had approximately the same activity as the most active form of copper chromite tested.
7.  $\text{V}_2\text{O}_4$  was found to be the most effective promoter for a  $\text{Cu}_2\text{O}-\text{CaO}$  catalyst.
8.  $\text{V}_2\text{O}_4$  was found to be the most effective promoter for a  $\text{CuO}-\text{CaO}$  catalyst.
9.  $\text{V}_2\text{O}_4$  was found to be a very effective promoter for a  $\text{Cu}-\text{CaO}$  catalyst.
10. A  $\text{Cu}_2\text{O}-\text{V}_2\text{O}_4-\text{CaO}$  catalyst was found to be effective for the hydrogenation of ketones.
11. The bright red color of inactivated copper oxide catalysts was not due to the formation of cuprous oxide but to the formation of metallic copper.

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# A MICROSCOPIC METHOD FOR MEASURING SOIL PORE SPACE AND FOR CHARACTERIZING SOIL STRUCTURE<sup>1</sup>

C. LOYAL W. SWANSON

*From the Department of Agronomy, Iowa State College*

Received June 10, 1941

It is generally accepted that the structure of the soil exerts a controlling effect on air and moisture movements within the soil body. There has also been much importance attached to the influence of soil structure on plant growth, infiltration, soil erosion, tilth, and other soil properties. Since pore space in soils is directly related to soil structure, every kind of soil structure has its own characteristic pore space. Natural soil structure cannot be adequately described without taking into consideration the sizes, numbers, shapes, orientations, and volumes of the various pores and without the use of some expression descriptive of the complete natural pattern of soil material and pore space. Thin sections prepared from oriented samples of undisturbed soil were found to show the natural soil-structure, pore-space pattern excellently.

The purpose of this investigation has been to develop a quantitative method by which the shape, size distribution, and volume of the larger pores could be measured in a sample of soil in its natural state. A micrometric method employing an adaptation of the Rosiwal technique for the volume-percentages of minerals in thin sections of rocks was applied to the measurement of pore space in thin sections of soil. This method was used in studying the pore-space relationships of the larger-sized pores (noncapillary range) of three soil profiles and of a virgin and a cultivated surface soil horizon.

The adapted Rosiwal method, called the micrometric method, was used in comparing the structural differences between the Marshall silt loam and Shelby silt loam soil profiles. The fact that a relatively large number of larger sized pores are found in the A<sub>3</sub> horizon of the Marshall silt loam as well as in the surface layer helps explain that soil's high infiltration rate. Strikingly shown in the Shelby is the high density of the B horizon and the decrease in the sizes and amounts of noncapillary pores with an increase in profile depth. The numerical values for volume-percentages of pore space at different depths in the two horizons are given.

It was found that a systematic method of selecting microscopic fields for measuring pore spaces in a thin section gave a more accurate estimate of the general mean than did random sampling. In the soils studied, there was no measurable orientation of pore space in either the plane of the soil horizon or in a plane perpendicular to the horizons. There was greater variability between individual sampling cylinders, using a 3 mm. by 3

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mm. sampling, than when a 1 mm. by 2 mm. sampling interval was employed.

The amount of work involved is not so great as to preclude use of the method as a means of characterizing structure in soil profiles. The choice of sampling interval used will be determined by the degree of accuracy which is desired. Differences in pore space between samples can be ascertained by the measurement of 50 to 100 microscopic fields per thin section on relatively few thin sections from each sample. Finer differences can be obtained by the micrometric analysis of more fields per thin section.

An advantage of the micrometric procedure is that it permits actual observation of the numbers, sizes, shapes, volumes, and distribution of the pores. The method allows the qualitative estimation of the natural pore space in samples of undisturbed soil, a measurement necessary for the correct appraisal of the relationships between other natural phenomena and soil pore space. The results of the sampling studies show that a quantitative expression of pore spaces can be obtained by a micrometric analysis.

The relationship of the structure of air-dried soils to wetted soils has been brought out and is graphically shown. The swelling of soils upon being wetted has been suggested as a factor of great importance in the infiltration of water into soils.

It was concluded that the method of wetting up soil cores and determining the soil pore-space relationships by the specific gravity volume-weight method gave data which were dependent on arbitrary factors, such as height of the soil column and time of wetting of the soil cores. This method furnished only information regarding the total, capillary, and noncapillary pore space in soils.

An apparatus was devised which made possible the quick and easy tracing of projected microscopic images of soil in the natural state as they appear in thin sections. It also facilitated the comparison of pore spaces on the basis of shape, size distribution, and volume.

Whether or not the pores are continuous in nature is believed to be another factor influencing infiltration and other soil properties. Some idea of the extent of this property may be had from a thin section study of a soil, and it may be shown by microprojection tracings. These tracings may be compared with those of the other horizons in the profile or with other profiles.

The pore-space relationships of a cultivated and a virgin soil horizon were studied. The results showed that the virgin soil had twice the volume of pores and a higher percentage of large pores than did the cultivated soil.

The micrometric method of determining pore space was compared with a moisture-tension method. The moisture-tension method yielded pore values comparable to those obtained by the micrometric method for a cultivated and a virgin soil.

It was shown that a knowledge of the distribution of pore space in natural soils is as important, if not more so, than of the total amount.

The micrometric method is another tool or method for studying the

structural relationships of natural soils. Combined with soil moisture studies, it provides additional means for the interpretation and understanding of natural soil phenomena. The measurement of volume-percentage pore space may be considered as an attempt to evaluate numerically the variable soil characteristic of natural pore space which affects permeability, tilth, air movements, and other soil factors.

# THE APPROXIMATE SOLUTION OF LINEAR DIFFERENTIAL EQUATIONS BY THE USE OF FUNCTIONALS<sup>1</sup>

CHARLES J. THORNE

*From the Department of Mathematics, Iowa State College*

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A general functional method is used to obtain an approximate solution to the physical problem represented by an infinite corner-loaded plate supported on an elastic foundation. The reaction of the foundation is assumed to satisfy Hooke's law.

The differential equation and boundary condition to which a solution must be obtained are:

$$(1) \quad \Delta^4 w = -\frac{k}{N} w,$$

$$(2) \quad M_x = -N \left[ \frac{\partial^2 w}{\partial x^2} + v \frac{\partial^2 w}{\partial y^2} \right]_{x=0} = 0,$$

$$(3) \quad M_y = -N \left[ \frac{\partial^2 w}{\partial y^2} + v \frac{\partial^2 w}{\partial x^2} \right]_{y=0} = 0,$$

$$(4) \quad R_x = -N \left[ \frac{\partial^3 w}{\partial x^3} + (2-v) \frac{\partial^3 w}{\partial x \partial y^2} \right]_{x=0} = 0,$$

$$(5) \quad R_y = -N \left[ \frac{\partial^3 w}{\partial y^3} + (2-v) \frac{\partial^3 w}{\partial x^2 \partial y} \right]_{y=0} = 0,$$

$$(6) \quad P_c = 2(Mxy)_c = -2N(1-v) \frac{\partial^2 w}{\partial x \partial y} \Big|_{y=0} = P.$$

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<sup>1</sup>Original thesis submitted June, 1941. Doctoral thesis number 631.

In which

$w$  = deflection of the plate,

$$N = \frac{EI}{1-\nu^2},$$

$$\Delta^4 = \frac{\partial^4}{\partial x^4} + 2\frac{\partial^4}{\partial x^2 \partial y^2} + \frac{\partial^4}{\partial y^4}.$$

$E$  = Young's modulus for material in plate,

$I$  = moment of inertia of unit area of cross section,

$\nu$  = Poisson's ratio for material in plate,

$k$  = Hooke's law constant for the foundation.

Here  $-kw$  represents the reaction of the foundation. The coordinate system is chosen so that the corner load  $P$  is at the origin and the edges of the plate lie along the axes.

The approximate solution used is

$$\begin{aligned}
 w = & e^{-(x+y)} [A_1 + A_2(x+y) + A_3(x^2+y^2) + A_4xy \\
 & + A_5(x^3+y^3) + A_6(x^2y+xy^2) + A_7(x^4+y^4) \\
 (7) \quad & + A_8(x^3y+xy^3) + A_9x^2y^2 + A_{10}(x^5+y^5) \\
 & + A_{11}(x^4y+xy^4) + A_{12}(x^3y^2+x^2y^3) + A_{13}(x^6+y^6) \\
 & + A_{14}(x^5y+xy^5) + A_{15}(x^4y^2+x^2y^4) + A_{16}x^3y^3]
 \end{aligned}$$

where  $x = \lambda_1 x$ , and  $\lambda_1 = \sqrt[4]{\frac{k}{4N}}$ .

The general functional method for problems of this type may be outlined as follows: we have the linear differential equation

$$(8) \quad L(w) = f(x_1, x_2, x_3, \dots, x_n)$$

and the  $b$  linear boundary conditions

$$(9) \quad B_l(w) = g \quad \text{when } \lambda_l = 0 \\ , \quad (l = 1, 2, \dots, b)$$

where the boundary equations are

$$(10) \quad \lambda_l \equiv \lambda_l(x_1, x_2, \dots, x_n) = 0.$$

The given differential operators are linear

$$(11) \quad L \equiv L(x_1, x_2, \dots, x_n, \frac{\partial}{\partial x_1}, \dots, \frac{\partial}{\partial x_n}, \dots, \frac{\partial^n}{\partial x_n^n})$$

$$\text{and } B_l \equiv B_l(x_1, x_2, \dots, x_n, \frac{\partial}{\partial x_1}, \dots, \dots)$$

It is assumed that  $w$  can be represented as a linear sum of expansion functions,  $w = a_i \Phi_i$ , where the  $a$ 's are constants to be determined and the  $\Phi$ 's are known expansion functions. Substitution of a finite number of terms of the assumed approximation into (8) and (9) results in expressions which are approximately equal to zero. The values of the  $a$ 's are determined by applying to these expressions functional families  $F_j$  for (7) and  $G_j$  for (8). In order to determine the constants of (7) the following functional families were chosen: for (1)

$$(12) \quad F_j \equiv \int_0^\infty \int_0^\infty x^\alpha y^\beta [ \quad ] dx dy$$

for  $(\alpha, \beta) = (0,0), (0,1), (1,1), (1,2), (2,2), (2,3), (3,3)$ . Because of the symmetry of (7), (2) and (3) will express the same analytical condition on the  $A$ 's; (4) and (5) will likewise be identical functions of the  $A$ 's. For application to both (2) and (4)

$$(13) \quad G_j \equiv \int_0^\infty y^\beta [ \quad ] dy$$

with  $\beta = 0, 1, 2, 3$ .

When the resultant set of linear equations is solved for the A's, the following values result:

$$\begin{aligned}
 A_1 &= \frac{.134974 + .051979v + .002016v^2 - .005152v^3 + .001727v^4 + .000033v^5}{D_1}, \\
 A_2 &= \frac{.0332455 - .0195173v + .0055404v^2 - .0097588v^3 + .0004063v^4 + .0000089v^5}{D_2}, \\
 A_3 &= \frac{.0751939 + .0077260v - .0151718v^2 + .0037443v^3 - .0020196v^4 + .0000158v^5 + .00000037v^6}{D_3}, \\
 A_4 &= \frac{-.0280910 - .0519321v + .0595263v^2 + .0108375v^3 + .0019643v^4 + .0000340v^5}{D_4}, \\
 A_5 &= \frac{-.0412761 + .0919599v - .0034287v^2 + .0043375v^3 - .011687v^4 - .0015493v^5 - .0000285v^6}{D_5}, \\
 A_6 &= \frac{.0176373 - .0022208v - .0250028v^2 + .0023520v^3 - .0096631v^4 - .0007150v^5 - .0000098v^6}{D_6}, \\
 A_7 &= \frac{.0059870 - .0574790v + .0061436v^2 - .0023870v^3 + .0085722v^4 + .0009716v^5 + .0000155v^6}{D_7}, \\
 A_8 &= \frac{.00129492 + .00620424v - .00488385v^2 - .00205360v^3 + .00327297v^4 + .00048193v^5 + .00001065v^6}{D_8}, \\
 A_9 &= \frac{-.0197480 + .0038150v + .0249330v^2 - .0022778v^3 + .0023597v^4 - .0002711v^5 - .0000101v^6}{D_9},
 \end{aligned}$$

$$A_{10} = \frac{- .00010134 + .00969721v - .00139865v^2 + .00037122v^3 - .00154854v^4 - .00015789v^5 - .00000219v^6}{D_1},$$

$$A_{11} = \frac{- .06126703 - .00055439v + .00243981v^2 + .00024974v^3 - .00035865v^4 - .00009616v^5 - .00000298v^6}{D_2},$$

$$A_{12} = \frac{.00286173 - .00155052v - .00339508v^2 + .00046645v^3 - .00029544v^4 + .00002956v^5 + .00000158v^6}{D_3},$$

$$A_{13} = \frac{- .000015446 - .000464914v + .000082814v^2 - .000017013v^3 + .000077083v^4 + .000006871v^5 + .000000074v^6}{D_4},$$

$$A_{14} = \frac{.000095681 - .000006494v - .000174971v^2 - .000007272v^3 + .000012005v^4 + .000006107v^5 + .000000235v^6}{D_5},$$

$$A_{15} = \frac{.000080748 + .000190622v + .000012940v^2 - .000033353v^3 + .000010692v^4 + .000011958v^5 + .000000240v^6}{D_6},$$

$$A_{16} = \frac{- .000237970 - .000090650v + .000449172v^2 - .000007165v^3 + .000015105v^4 - .000030166v^5 - .0000000822v^6}{D_7},$$

$$\text{where } Dv = .229556 - .039081v - .050461v^2 - .025203v^3 - .002878v^4 - .000049v^5 \text{ and } \Theta v = \frac{P}{2N(1-v)2_1^2}$$

Numerical values of these A's for  $v = .15, .2, .25$ , and  $.3$  are given. The values of the A's in terms of an arbitrary  $\lambda_1$  are given for  $v = .2, .25$ , and  $.3$ . Indications of the error are discussed.

MONOGRAPH OF THE PSYLLINAE AND TRIOZINAE  
(PSYLLIDAE: HOMOPTERA) IN AMERICA  
NORTH OF MEXICO<sup>1</sup>

LEONARD D. TUTHILL

From the Department of Zoology and Entomology, Iowa State College

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Included in this taxonomic treatise on the psylline and triozine Psyllidae of Continental America north of Mexico, in addition to the systematic treatment, are a general discussion of the morphology and biology of the group, a discussion of methods of collecting and preserving, an explanation of the terms and methods used, and a bibliography listing all of the papers concerned with North American forms and the important ones dealing with psyllids in other areas of the world.

Keys are given to the genera of both subfamilies and to the species of each genus. The family and subfamilies are defined, and synonymy and nomenclatorial notes given. The genera are described, and synonymy is given.

In addition to a description of each species the complete synonymy and, except where too voluminous, a complete list of citations, the known range, the host plant or plants, any biological data available, and the location of the types, where known, are included.

Especial attention is given to the economically important species, chiefly *Paratrioza cockerelli*, *Psylla pyricola* and *Psylla mali*.

There are seven plates of figures showing characters of the new species and of some species of which no figures have been available heretofore.

Eight genera of Psyllinae are listed: *Psylla* (53 spp.), *Arytaena* (14 spp.), *Psyllopsis* (3 spp.), *Euphalerus* (7 spp.), *Euphyllura* (8 spp.), *Pachypsylla* (7 spp.), *Tetragonocephala* (1 sp.), and *Spanioneura* (1 sp.). A total of ninety-four species and varieties are included, sixteen of which are described as new.

In the Triozinae also there are eight genera: *Trioza* (40 spp.), *Paratrioza* (7 spp.), *Neotriozella* (5 spp.), *Metatrioza* (1 sp.), *Leuronota* (2 spp.), *Ceropsylla* (1 sp.), *Hemitrioza* (2 spp.), and *Levidea* (1 sp.). A total of fifty-nine species and varieties are listed, four of which are described as new.

<sup>1</sup>Original thesis submitted June, 1941. Doctoral thesis number 607.

# SOME INTERNAL EFFECTS OF DINITROPHENOLS ON INSECTS<sup>1</sup>

GETULIO B. VIADO

From the Department of Zoology and Entomology, Iowa State College

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Although the effectiveness of dinitrophenols as insecticides has long been recognized, the literature on this subject indicates that it was not until the past five years that the extensive use of these compounds in the control of various insects has been studied. Nearly all the literature on the use of these chemicals as insect toxicants relates to the practical value of these substances as insecticides. Very little is known about the action of these compounds on insects. Studies were conducted by the writer to determine the effect of 3,5-dinitro-o-cresol and 2,4-dinitro-6-cyclohexylphenol on the larvae of a species of blowfly, *Cynomyia cadaverina* Desvoidy, and housefly, *Musca domestica* Linn. and the adults of the American cockroach, *Periplaneta americana* Linn.

Methods were devised for the study of these chemicals as to their repellent action on the blowfly and housefly larvae, toxicity to the housefly larvae and pupae, effect on the American cockroach when solutions of these compounds are injected into the insects and when given to the insects through the oral cavity, and their effect on the American cockroach when applied as undiluted dusts on various parts of the bodies of the insects. The effects of 2,4-dinitro-6-cyclohexylphenol on the hemolymph cell counts of the blowfly larvae when the poison is mixed with the insect food was also studied. Various colorimetric methods for the detection of these dinitrophenols were tried in the detection of minute quantities of these compounds in the body of the insects. An attempt was made by the writer to differentiate between the two undiluted dinitrophenols in a micro-qualitative way with the use of common laboratory reagents. Descriptions of the various methods used in this investigation are described.

The results of the investigation indicate that:

1. 3,5-dinitro-o-cresol and 2,4-dinitro-6-cyclohexylphenol can be readily distinguished in the uncombined state through the use of a micro-qualitative method involving common laboratory reagents.
2. Neither the Fischer nor the Fleck colorimetric method for detecting the presence of dinitrocresol give very satisfactory results when used for the detection of minute quantities of this compound in the insect body. The Meyer-Drutel method for the colorimetric determination of 2,4-dinitrophenol in urine, likewise, cannot be successfully employed in this particular type of work.
3. No decided effect of 2,4-dinitro-6-cyclohexylphenol on the hemolymph cells of 3-day-old larvae of *Cynomyia cadaverina* Desvoidy, was

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noted. The cells of both the treated and untreated larvae appeared identical. Cell counts made on the treated and untreated lots were treated statistically, but the difference between the means of the lots was not significant, the value of  $t$  obtained being only 1.74 with 222 degrees of freedom.

4. The toxicity of solutions of these compounds on the American cockroach when injected into the insect or taken through the oral cavity by drinking can be studied quantitatively. The methods employed are described.

5. The first visible indication of a major effect of the poisons on the American cockroach is paralysis. When the insects are dusted on all parts of the body, paralysis progresses from the anterior to the posterior part of the body.

6. The behavior of the blowfly and housefly larvae and the nature of the tracheae in the antennae of the cockroaches treated with the dinitrophenols indicate that something besides nerve poisoning may be concerned.

7. These compounds are repellent to the larvae of the housefly and blowfly.

8. The 3,5-dinitro-o-cresol is more toxic to the insects than the 2,4-dinitro-6-cyclohexylphenol.

9. The housefly larvae are more resistant to these poisons than the blowfly.

10. When solutions of the ammonium and sodium salts of the two dinitrophenols are taken either with the food or imbibed in solution, they are toxic to the insects, indicating that they are stomach poisons.

11. There exists an individual variation among the insects used in this investigation as to their susceptibility to the poisons.

# THE EFFECT OF SOYBEANS AND SOYBEAN OIL ON MILK AND BUTTERFAT PRODUCTION AND ON THE QUALITY OF THE BUTTERFAT<sup>1</sup>

NEIL KENNETH WILLIAMS

*From the Laboratory of Dairy Husbandry, Department of Animal Husbandry,  
Iowa State College*

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In a series of four experiments conducted at the Iowa Agricultural Experiment Station, a study was made of the effect of feeding soybeans and soybean oil to dairy cattle with particular reference to milk and butterfat yields and to certain chemical and physical characteristics of the butterfat.

## *First Trial.*

In the first experiment eight cows were used in a paired-feeding trial, one group of which was fed a normal ration of alfalfa hay, silage and mixed grains, while the other, following a preliminary feeding period was successfully carried for 22 weeks on a experimental ration of soybeans (grain) and silage. The soybeans were fed at the rate of 1 pound per 5 pounds of milk produced. Up to 9 pounds daily of soybeans were fed. The silage, which was fed *ad libitum*, was corn silage, sorghum silage, or sorghum silage supplemented with 1 pound of corn per 20 pounds of silage and was consumed at about the rate of 6 pounds daily per 100 pounds live weight. Bone meal and salt were also provided. No indication of vitamin A or other deficiencies were observed. The cows very nearly maintained their weight during the experimental feeding period.

As compared with the cows on the normal grain ration the cows on the experimental ration produced slightly less milk which had a higher fat percentage and yielded slightly more butterfat. These differences in milk and fat yields were nonsignificant statistically, while the differences in percentage of butterfat were highly significant. Analysis of the milk and butterfat showed no difference in the percentage of protein in the milk, and marked differences which were highly significant in the nature of the butterfat as indicated by the iodine, Reichert-Meissl, and Polenske values. The first value was raised and the other two lowered by the experimental feeding. The experimental ration had no particular effect on milk flavor or on specific gravity of the milk.

It was concluded that under suitable price conditions the experimental ration was practical for cows giving up to 50 pounds of milk daily. With higher producing cows a slight modification might be necessary.

<sup>1</sup> Original thesis submitted July 17, 1939. Doctoral thesis number 543.

### Second Trial.

In the second experiment two groups of cows were used in a paired-double-reversal trial of three experimental periods in which the roughage (alfalfa hay and corn silage) and grain mixture of the two rations used were identical throughout except that one carried soybean oil which was replaced by an isodynamic amount of starch in the second grain mixture. The grain mixtures, which also included corn, oats, and cracked soybeans, were compounded and fed at such a rate that the cows received in their grain, respectively, 94 per cent and 41 per cent as much fat as they secreted in their milk.

As compared with the medium (41 per cent) fat ration, during the last five weeks of each six-weeks' period, the feeding of the high (94 per cent) fat ration increased milk production 5.96 pounds per cow per week or 2.17 per cent. The percentage of butterfat in the milk decreased from 3.37 to 3.17. This caused a decrease in total butterfat production of 0.36 pound per cow per week or 3.92 per cent. Of these changes, only the percentage of butterfat was statistically significant.

The feeding of the high fat ration increased the iodine value of the butterfat 11.65 numbers and lowered the Reichert-Meissl and Polenske values 5.55 and 1.04 numbers, respectively, when compared with those obtained from the medium fat ration. All of these changes were highly significant statistically. The live weights of the cows were not affected by the type of feeding.

When the data collected during the experimental periods are compared with those collected during the preliminary and post-experimental periods when a herd grain ration was fed, and with some data obtained from a third group of cows on a herd ration, it is evident that most of the changes in milk quality occurring in the experimental periods were due to the high fat rather than the medium fat ration. Therefore, the changes observed were due almost entirely to the feeding of the free soybean oil and not to the oil that was in the beans.

The high fat ration increased the time required for churning the cream and caused the production of an oily butter, even though it also raised the melting point of the butterfat. The color of the butter was reduced until it was very nearly white. The medium fat ration also somewhat reduced the color of the butter.

Some of the cows consumed nearly 2 pounds of fat daily in the grain mixture without any apparent ill effect.

By comparing the iodine value of the soybean oil and the changes occurring in the iodine value of the butterfat, it was found that, at least as far as unsaturation was concerned, the effect of feed fat on milk fat was the same as if 23 per cent of the soybean oil fed had been mixed with the original butterfat.

### Third Trial.

In the third experiment two groups of cows were used in a paired-

double-reversal trial with three experimental periods of four weeks each. Throughout the trial the cows were fed timothy hay, instead of alfalfa as in the previous trial, and corn silage in order to keep the protein intake more nearly balanced with actual requirements. One grain ration contained 77.8 per cent soybeans and was fed at such a rate that the cows received in their grain 105 per cent as much fat as they secreted in their milk. The second grain ration was identical except that part of the soybeans were replaced by soybean oil meal from beans of the same source and processed at a low temperature so as to avoid altering the biological value of the proteins. Starch was also present in an amount isodynamically equivalent to the difference in the soybean fat content between the two rations. The second ration was fed at such a rate that the cows received in their grain 48 per cent as much fat as they secreted in their milk.

Compared with the medium fat ration the high fat ration decreased milk production 10.6 pounds per cow per week (3.50 per cent), increased butterfat production 0.73 pound per cow per week (6.92 per cent), and increased the percentage of butterfat in the milk 0.38 (10.98 per cent). All of these changes were statistically significant or highly significant. The increased butterfat production amounted to 12.1 per cent of the extra fat fed. Up to 2.7 pounds of grain fat (mostly as part of the soybeans) were fed to cows daily without ill effects. The cows lost 5.8 pounds in weight while on the high fat ration.

As compared with the herd ration, the effects of the high fat ration were even greater.

Both rations increased the iodine and thiocyanogen-iodine values of the butterfat as well as the percentages of oleic and linoleic (or its optical isomer) acids, and lowered the Reichert-Meissl and Polenske values from those obtained when the herd ration was fed. The high fat ration caused greater changes than the lower fat ration, with the differences between the rations all highly significant. The high fat ration also increased the melting point of the butterfat about 2° C.

Both rations shortened the time required for churning and greatly reduced the color of the butterfat. This reduction in color was an advantage in the making of "Blue Cheese." The high fat ration also increased the melting point of the butterfat about 2° C.

The effect on the unsaturation of the butterfat caused by the soybeans that were fed was the same as if 11.4 per cent of the fat in the soybeans had been mixed with the original butterfat. This is only half the value obtained when soybean oil was fed.

By comparison with previous experiments it was concluded that the method of feeding soybean fat—in the bean or as oil—caused entirely different reactions in milk and butterfat production. There was also a difference in the degree of deviation of the various constants determined, the maximum deviations from the original factors which were obtained during the preliminary feeding period being caused by the feeding of the soybean oil.

*Fourth Trial.*

Two groups of cows were used in a paired-double-reversal trial of three four-week periods. The rations compared were composed of alfalfa hay, corn silage, and a grain mixture containing 50 per cent by weight of soybeans, and were identical with each other except that the soybeans in one grain ration were replaced in the second grain ration by soybean oil and soybean oil meal manufactured at a low temperature so as not to influence the biological value of the protein. A selected lot of soybeans, one-half of which was extracted, was the source of the soybeans, soybean oil meal, and soybean oil used in this trial. The cows on either ration received 80 per cent as much fat in their grain as they secreted in their milk.

The ration containing the soybeans caused the milk production to decrease 7.8 pounds per cow per week (2.38 per cent), the percentage of butterfat in the milk to increase 0.33 (11.11 per cent), and total butterfat production to increase 0.83 pound per cow per week (8.56 per cent) as compared with the ration having grain containing soybean meal and soybean oil. When these yields were compared with those obtained when the cows were fed the regular herd ration, it was noted that the ration having the unprocessed soybeans decreased milk production and increased butterfat production, while the ration containing free soybean oil increased milk production but decreased butterfat production. Statistically, these changes were highly significant or significant. The live weights of the cows were relatively unaffected by the feeding of the experimental rations.

Both rations greatly affected the nature of the butterfat. The greatest rise in iodine and thiocyanogen-iodine values and decrease in Reichert-Meissl and Polenske values was caused by the feeding of the ration containing free soybean oil. The percentage of oleic acid was also greatest when this ration was fed. The percentage of linoleic acid (or its optical isomer) in the butterfat was greatest when the soybean ration was fed. These differences were highly significant.

Both rations increased the melting point of the butterfat and decreased the color.

In general, feeding the ration containing unprocessed soybeans decreased churning time, while feeding the ration containing free soybean oil increased it.

This experiment conclusively demonstrated that the method of feeding soybean fat has an important effect on the yield of milk and the quantity and quality of its butterfat.

# ' ACTION OF MOLD INHIBITORS ON DAIRY PRODUCTS<sup>1</sup>

JUDDIE JOHNSON WILLINGHAM

From the Department of Dairy Industry, Iowa State College

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Growth of molds on dairy products is a source of loss to the dairy industry, particularly with butter and various cheeses. The studies reported herein were undertaken to determine the mold-inhibiting properties of various compounds, especially propionic acid and its salts.

The samples of salted and unsalted butter (with the exception of those churned from raw cream), the tubes of Czapek's medium, and some of the samples of cheddar cheese were inoculated with mold suspensions prepared from portions of moldy butter or moldy cheese, from plates poured for mold counts on butter, and from molds on the shelves of a cheese-curing room. All the other samples were not inoculated since they contained molds.

Propionic acid was very effective in inhibiting mold growth. With raw milk and raw cream, a concentration of 0.5 or 1 per cent prevented mold growth, and in Czapek's medium 1 per cent prevented growth. Wrapping salted and unsalted butter in parchment dipped in a 5 per cent solution of propionic acid and dipping cheddar cheese in a 5, 7, or 10 per cent solution inhibited mold growth. Smearing the surface of cottage cheese with a 7 per cent solution was ineffective, but mixing the solution with the cheese delayed mold growth. With pure cultures of *Penicillium roqueforti*, *Penicillium camemberti*, and *Oospora lactis* in Czapek's medium, a 0.5 or 1 per cent concentration delayed growth.

Calcium propionate was less effective than propionic acid. With raw cream, a concentration of 0.8 or 1 per cent inhibited mold growth, and with raw milk and Czapek's medium, 5 per cent was effective. With salted and unsalted butter, dipping the butter or dipping parchment for wrapping the butter in a 12.5, 15, 18, 19, or 25 per cent solution inhibited growth. Dipping cheddar cheese in a 10 or 12 per cent solution gave some inhibition, and an 18 or 25 per cent solution was effective. Dipping swiss-type cheese in an 18 per cent solution delayed growth. Parchment treated commercially with 6, 10, 25, or 30 per cent calcium propionate was used to wrap butter and cheese and gave some inhibition but was less effective than dipping the butter or cheese or dipping parchment for wrapping the products in solutions of calcium propionate. Smearing the surface of cottage cheese with an 18 per cent solution did not delay growth, but mixing the solution with the cheese was effective. A 5 per cent solution inhibited growth of pure cultures of *P. roqueforti*, *P. camemberti*, and *O. lactis* in Czapek's medium.

<sup>1</sup>Original thesis submitted June, 1941. Doctoral thesis number 605.

Sodium propionate was less effective than calcium propionate or propionic acid. With raw cream, a 1 per cent concentration inhibited growth, and with raw milk and Czapek's medium, a 5 per cent concentration was effective. Dipping salted and unsalted butter in an 18 or 25 per cent solution inhibited growth. Dipping cheddar cheese in a 12 per cent solution gave some inhibition, and an 18 per cent solution was effective. Smearing the surface of cottage cheese with an 18 per cent solution was ineffective, but mixing the solution with the cheese inhibited growth. A solution of 1 or 5 per cent was ineffective with pure cultures of *P. roqueforti* and *O. lactis* in Czapek's medium but inhibited growth of *P. camemberti*.

Acetic acid and calcium acetate used in a concentration of 1 per cent or less with raw cream did not inhibit mold growth.

Sodium benzoate in a 1 per cent solution was effective as a mold inhibitor when used in raw cream and Czapek's medium. A number of compounds that have been suggested for the destruction of bacteria under various conditions were unsatisfactory as mold inhibitors when used with cream, Czapek's medium, butter, and cheese.

The use of 1 per cent of various wetting agents, along with the mold inhibitors, increased the effectiveness of the inhibitors with liquids, such as cream and Czapek's medium, but did not increase the effectiveness with solid materials, such as butter and cheese.

The use of special wax parchments instead of regular parchment for wrapping butter and cheese did not increase the mold-inhibiting properties of propionic acid and its salts. The use of special cellophane for wrapping cheese increased the effectiveness of the mold inhibitors, as compared with parchment and tin foil.

The samples of butter and cheese held at 15°C. usually showed mold growth before the samples held at 10°C. Unsalted butter commonly showed mold growth before salted butter. The use of different dilutions of a mold suspension to inoculate butter made little difference in the period before mold growth appeared. There was little difference between dusting mold spores on the surface of a medium containing propionic acid or its salts and distributing them through the medium in the time required for mold growth to be evident.

With 1 per cent propionic acid in inoculated milk and raw cream, the effect of the mold inhibitor was evident microscopically by the disappearance of the spores from the microscopic preparations. With lower concentrations of propionic acid or with a 1, 2, or 5 per cent concentration of calcium propionate, there was a delay in the germination of the spores and often a disappearance of some spores. When germination occurred, the growth appeared to lack vigor.

With calcium propionate in inoculated milk and raw cream, lowering the pH with lactic acid increased the inhibition of the molds.

Propionic acid, calcium propionate, and sodium propionate imparted a slight odor and flavor to the dairy products, but neither was objectionable, and with cheese the flavor was rather pleasing. White discolorations on

butter and cheese were sometimes produced but disappeared after a few days. Acetic acid produced a slight odor. The other compounds produced no noticeable odors, flavors, or discolorations.

Since the conditions in many of the trials were very similar to conditions in commercial plants, it appears that propionic acid and its salts would be useful commercially for preventing mold growth on dairy products.

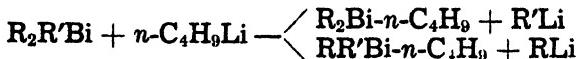
# UNSYMMETRICAL ORGANOBISMUTH COMPOUNDS<sup>1</sup>

HARRY LOUIS YABLUNKY

From the Department of Chemistry, Iowa State College

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The metal-metal interconversion reaction between triarylbismuth compounds and *n*-butyllithium<sup>2</sup> has been extended to determine the relative labilities of aryl radicals attached to bismuth in unsymmetrical organobismuth compounds.



The reactions were allowed to proceed in diethyl ether for 10 minutes at 25° C., and the amounts of RLi and/or R'Li formed were estimated subsequent to carbonation with dry ice as the corresponding carboxylic acids.

Di-*p*-chlorophenyl-*o*-tolylbismuth yielded 38.4 per cent *p*-chlorobenzoic acid and no *o*-toluic acid; di-*p*-chlorophenyl-*a*-naphthylbismuth, 21.9 per cent *p*-chlorobenzoic acid, and 22.7 per cent *a*-naphthoic acid; di-*p*-chlorophenyl-*p*-tolylbismuth, 28.2 per cent *p*-chlorobenzoic acid, and 7.7 per cent *p*-toluic acid; diphenyl-*p*-chlorophenylbismuth, 29.4 per cent *p*-chlorobenzoic acid and 10 per cent benzoic acid; di-*p*-tolyl-*a*-naphthylbismuth, 6.1 per cent *a*-naphthoic acid and no *p*-toluic acid; and diphenyl-*a*-naphthylbismuth, 21.6 per cent *a*-naphthoic acid and 1.7 per cent benzoic acid.

When a similar interconversion was carried out between symmetrical  $R_3Bi$  compounds and *n*-butyllithium under corresponding conditions, tri-*p*-chlorophenylbismuth gave 48.6 per cent *p*-chlorobenzoic acid; tri-*p*-bromophenylbismuth, 41.3 per cent *p*-bromobenzoic acid; tri-*p*-fluorophenylbismuth, 41.4 per cent *p*-fluorobenzoic acid; triphenylbismuth, 5.8 per cent benzoic acid; tri-*p*-tolylbismuth, 2.2 per cent *p*-toluic acid; and tri-*p*-ethoxyphenylbismuth, 2.0 per cent *p*-ethoxybenzoic acid. Tri-*o*-tolylbismuth, tri-*o*-chlorophenylbismuth, and trimesitylbismuth underwent no metal-metal interchange with *n*-butyllithium under the conditions described above. With tri-*a*-naphthylbismuth, the yield of *a*-naphthoic acid was 1.2 per cent, due probably to the insolubility of this bismuth compound in ether. In all the other metal-metal interconversions described above, the bismuth compounds were dissolved completely in the ether (40-60 ml.) prior to the addition of *n*-butyllithium.

When metal-metal interconversions were carried out between  $R_2Hg$  and  $RR'Hg$  compounds and *n*-butyllithium under comparable conditions, di-*p*-chlorophenylmercury gave 67.8 per cent *p*-chlorobenzoic acid and phenyl-*p*-chlorophenylmercury, 54.4 per cent *p*-chlorobenzoic acid and

<sup>1</sup>Original thesis submitted December 18, 1940. Doctoral thesis number 598.

<sup>2</sup>Gilman, Yablunsky, and Svigoon, J. Am. Chem. Soc., 61, 1170 (1939).

4.9 per cent benzoic acid. The absence of any observable reaction between trimesitylbismuth and *n*-butyllithium was in direct contrast to the reaction with dimesitylmercury which gave 72.5 per cent of the corresponding carboxylic acid. Steric hindrance may play an important rôle in these reactions, and this explanation appears valid also for the absence of any reaction between tri-*o*-tolylbismuth or tri-*o*-chlorophenylbismuth and *n*-butyllithium. Furthermore, di-*p*-tolylmercury and *n*-butyllithium gave 41.5 per cent *p*-toluic acid, while diphenylmercury gave 26.1 per cent benzoic acid. Thus, the *p*-tolyl radical was more labile than phenyl when attached to mercury, and this was in contrast to the labilities of these radicals when attached to bismuth.

These latter experiments showed that the central metal of the organometallic molecule exerted an influence on the rate of metal-metal interconversion. In addition, the reaction between tri-*p*-chlorophenylbismuth and *n*-butyllithium which gave 48.6 per cent cleavage in diethyl ether gave no reaction in a mixture of petroleum ether (b.p., 28-30°) and benzene. Thus, the nature of the solvent played an important rôle in all these reactions. Di-*p*-tolylmercury and di-*p*-chlorophenylmercury, which are slightly soluble in ether, gave an increase in interconversion with an increase in volume of the solvent. This was due to the fact that more of the compound was in solution and, hence, available for reaction. Obviously, the solubility of the organometallic compound must be considered a factor in all these or related reactions.

Based on the metal-metal interconversion reactions between  $R_2R'Bi$  and  $R_3Bi$  compounds and *n*-butyllithium, it was possible to evolve the following series of radicals in order of decreasing lability:

*p*-chlorophenyl, *p*-bromophenyl, *p*-fluorophenyl,  $\alpha$ -naphthyl

phenyl  
*p*-tolyl  
*p*-ethoxyphenyl  
 $\alpha$ -tolyl, *o*-chlorophenyl, mesityl

In the series of radicals obtained by the cleavage of unsymmetrical organomercury<sup>3</sup> and other organometallic<sup>4</sup> compounds, the radicals under discussion possess the following order of decreasing lability:

mesityl, *p*-ethoxyphenyl<sup>5</sup>  
 $\alpha$ -naphthyl  
 $\alpha$ -tolyl

<sup>3</sup> For leading references to the work of Kharasch, see *J. Chem. Education*, 11, 82 (1934); *J. Org. Chem.*, 3, 409 (1938). See also, Whitmore and Bernstein, *J. Am. Chem. Soc.*, 60, 2628 (1938).

<sup>4</sup> Gilman, Towne, and Jones, *ibid.*, 55, 4689 (1938).

<sup>5</sup> The *p*-ethoxyphenyl radical is not listed in this series, but the *p*-methoxyphenyl radical is placed above the *o*-tolyl radical and approximately equal to the mesityl radical in lability. One may assume arbitrarily that the *p*-ethoxyphenyl group would occupy a position similar to the *p*-methoxyphenyl group.

p-tolyl  
p-fluorophenyl  
phenyl  
p-chlorophenyl, p-bromophenyl  
o-chlorophenyl

The metal-metal interconversion reaction between organobismuth compounds and *n*-butyllithium confirmed the position of the *p*-fluorophenyl radical in the series of radicals obtained by the cleavage of unsymmetrical mercurials by hydrogen chloride.<sup>6</sup> In addition, the cleavage with *n*-butyllithium showed that the *p*-bromophenyl and *p*-chlorophenyl radicals were also exceptions to the rule that direct substitution decreases the lability of the phenyl radical.<sup>7</sup>

The metal-metal interconversions between symmetrical triarylbismuth compounds and *n*-butyllithium were of more than ordinary interest. They offered confirmation of the series of radicals obtained by the metal-metal interchange reactions carried out with unsymmetrical bismuth compounds. This indicated that a series of radicals need not of necessity be derived from the preferential replacement of one radical from an unsymmetrical organometallic compound.

The two series of radicals given above differ considerably and are almost reversals of one another. The futility of trying to generalize any single series of radicals is evident. At this time it is unwise to say any more than that the series of radicals obtained by the metal-metal interconversion reactions with *n*-butyllithium is restricted in use to the reaction from which it was derived.

<sup>6</sup>Kharasch, Pines, and Levine, *J. Org. Chem.*, **3**, 347 (1938).  
<sup>7</sup>Kharasch and Flenner, *J. Am. Chem. Soc.*, **54**, 674 (1932).

# NUTRITIONAL STATUS OF IOWA STATE COLLEGE WOMEN.

## VI. FACTORS CONTRIBUTING TO VARIABILITY IN BASAL METABOLISM<sup>1</sup>

CHARLOTTE MARIE YOUNG

*From the Department of Foods and Nutrition, Iowa State College*

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At least four basal metabolism observations, two on each of two days, are reported on 169 different college women from 17 to 29 years of age, 149 of whom were between 17 and 23 years, inclusive. Of this original group, 55 individuals remained in college and were observed for more than one year: 55 for two years, 39 for three years, and 10 for the entire four-year college period. This group is known as the repeat group. The remaining 114 were observed one year only and are referred to as the non-repeats. Thus both a longitudinal and a cross-sectional study has been possible. This study is the most extensive college study so far reported.

The purpose of the study was to report the basal metabolism of the college woman measured under her usual living conditions with no restriction of her routine except that imposed by the post absorptive state. Variability is dealt with as a fundamental characteristic of basal metabolism. By statistical methods an attempt has been made to segregate some of the factors which may contribute to the variability of basal metabolism, for example, the effects of the amount of sleep the night previous to testing, the menstrual cycle, interindividual and intraindividual variability, the state of nutrition as measured by hemoglobin level, the method of selection of data, training in the testing routine, and fatigue. An endeavor has also been made to clarify, to some extent, the existing lack of parallelism between present standards and observed basal metabolism in college women. On the basis of this body of data a tentative standard for college women has been formulated.

The repeat and non-repeat groups were found to differ significantly in mean metabolism. The metabolism of the repeat group was less than half as variable as that of the non-repeat group. Consequently in all analyses the repeat and non-repeat data were treated as two separate series.

The average sleeping time the night previous to testing was 6.8 hours. Within the range studied, it made no difference in the basal metabolism observed on two successive days whether the tests were preceded by the same or different amounts of sleep.

The reported effects of the menstrual cycle on basal metabolism were confirmed. These consist essentially of a premenstrual rise in basal metabolism with a lowering during actual menstruation and in the imme-

<sup>1</sup> Original thesis submitted June 5, 1940. Doctoral thesis number 565.

diate postmenstrual period. Statistical analysis by this laboratory of Wakeham's data (1923) showed a highly significant increase in basal metabolism in the premenstrual period.

Standard deviations for total interindividual, interindividual, intra-individual, intradaily intraindividual, and day-to-day intraindividual variabilities are presented for both the repeat and non-repeat groups; these confirm the work of the Mayo Foundation. The data presented offer the advantage of obtaining all of the variability measures, both interindividual and intraindividual, from the same subjects, rather than from the three distinct and unequal groups used by the Mayo Foundation workers. Standard deviations for the non-repeat group suggest a greater variability in the basal metabolism of college women than in the clinic individuals surveyed by the Mayo group.

Using hemoglobin as an index, the nutritive state, within ranges which exclude the seriously abnormal, was unrelated to the level of basal metabolism.

It was found that training in the testing routine had a highly significant effect on basal metabolism, but apparently one day of testing was sufficient to acquaint the subject with the routine of the observation. For careful physiologic work, it is suggested that one practice day of testing be made before recording data. Since there is usually no provision for repeat testing in clinical studies, the effect of the newness of the test should be included in standards used for clinical purposes.

The method of selection of data proved to be a variability factor of some importance. For a satisfactory comparison of basal metabolism, the method of selection of data must be uniform. The method suggested by this laboratory as the procedure of choice is the use of all data, unless at the time of the test and before calculation of results the test is discarded for reasons of error in technic or non-basal condition of the subject. Using this method as a standard of comparison, the method of averaging the lowest observation on each of two days which check within 5 per cent and the method of using only the first day of observation appear to change the sample studied.

The hypothesis was advanced that the fatigue picture observed in very active college women is reflected in an increased day-to-day variability in their basal metabolism. Evidence is presented for this hypothesis.

Because of the very small variance between tests made on the same day, only one observation per day would seem to be necessary. Since the variance between days on the same individual was highly significant, observations on more than one day are essential to establish the basal metabolism of the individual for physiological studies.

There seems little need to explain the lowered basal metabolism reported for college women on the basis of a chronic low grade under-nutrition. The simplest interpretation offered so far is the dearth of data for this age group at the time standards were compiled, which resulted in prediction standards too high for physiological studies.

A tentative prediction curve for basal metabolism of women 17 through 28 years of age is proposed. The curve follows much the same shape as the Mayo Foundation curve for women of these ages, but at a definitely and almost constantly lower level. This lower level is in keeping with the basal metabolism observations reported on college women. It is suggested that the difference between the Mayo Foundation and the Iowa State standards may be due to the elevating effect of apprehension present in the clinical situation under which the Mayo Foundation standards were made, but which is absent in the Iowa State testing situation.

# THE NUMBER OF STEREOISOMERIC ALCOHOLS

EDWARD S. ALLEN AND HARVEY DIEHL

*From the Departments of Mathematics and Chemistry, Iowa State College*

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The problem of establishing the number of possible isomers of the alcohols of increasing carbon content has attracted the attention of various workers during the past sixty-five years. No general solution of the problem appears to be possible, but the brilliant attack by Henze and Blair<sup>1</sup> makes the calculation of the number of isomeric alcohols of any given carbon content from the number of those of fewer carbon atoms a not too arduous task. Going further and with a similar approach, Henze and Blair<sup>2</sup> devised formulas for calculating the number of stereoisomeric and non-stereoisomeric alcohols of a given carbon content, again, of course, presupposing such a knowledge for the alcohols of one less carbon content. By stereoisomeric was meant any compound capable of resolution into optically active forms, irrespective of the number of centers of asymmetry or the number of such isomers possible for a given structural isomer; non-stereoisomers are those simply lacking a center of asymmetry and for which, of course, only one isomer is known. The method thus counts all of the isomers, and distinguishes those which have asymmetric carbon atoms from those that do not, but does not state how many have two, three, etc. asymmetric carbon atoms.

We have now adapted the method of approach of Henze and Blair to a more general treatment of the problem which does yield this information. It has been necessary to introduce a factor, which for convenience is designated as the "asymmetry number" and to recast the notation of Henze and Blair in a somewhat more symbolic and condensed form.

The asymmetry number,  $\alpha$ , is the number of possible stereoisomeric forms of a given structural isomer. For all compounds having no center of asymmetry, it has the value 1; these are the non-stereoisomeric forms of Henze and Blair. For a structural isomer containing one asymmetric carbon atom,  $\alpha$  equals 2. For a structural isomer having two asymmetric carbon atoms,  $\alpha$  may be 3 or 4 depending on whether the groups surrounding the asymmetric atoms are the same in the two cases or not; where the groups are not the same,  $\alpha$  has the value 4, or in general, if  $n$  be the number of different asymmetric carbon atoms, the number of stereoisomers is  $2^n$ . Tartaric acid is, of course, the most common example of the other case, the asymmetric carbon atoms being surrounded by the same groups;  $\alpha$  has the value 3, corresponding to the *d*-, *l*-, and *meso*-forms. This situation cannot arise among the alcohols but does among the hydrocarbons and glycols.

<sup>1</sup> Henze, H. R., and C. M. Blair. *J. Amer. Chem. Soc.*, 53, 3042, (1931). See this paper also for a bibliography of earlier work.

<sup>2</sup> *J. Amer. Chem. Soc.*, 54, 1098 (1932).

For a given number  $n$  of carbon atoms, the number of structural isomers of any given asymmetry number is represented by  $T_{n,a}$  and the number of stereoisomers of any given asymmetry number is then  $T'_{n,a}$ , which is equal to  $aT_{n,a}$ . The total number of stereoisomers of all types for a given number of carbon atoms,  $T''_n$ , is then the sum of these  $T'_{n,a}$  values for values of  $a$  which occur for this  $n$ .

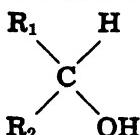
$$T''_n = \sum_a T'_{n,a}$$

The general process of counting the number of isomers for a given  $n$  consists in considering the groups attached to the carbon atom carrying the hydroxyl group and coupling them in all possible manners. In the method of development the primary, secondary, and tertiary alcohols are considered separately. The primary alcohols may be considered as being derived by the attachment of an alkyl group having one less carbon atom to the carbon atom carrying the hydroxyl group. The number of primary alcohols is then the same as the total number of alcohols of all types having one less carbon atom, for the hydroxyl group may simply be replaced in each of these by  $\text{-CH}_2\text{-OH}$ . Moreover, no change in asymmetry number occurs since no new asymmetric carbon atom is introduced. Symbolically, then,

### Type 1

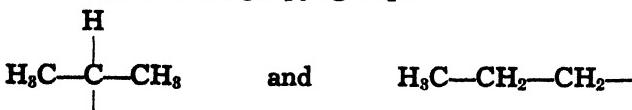
$$p_{n,a} = T_{n-1,a}$$

The secondary alcohols may be considered as being derived by the attachment of two alkyl groups,  $R_1$  and  $R_2$ , to the carbon atom carrying



and the isomerism of the alcohol.

thus represented will depend on the similarities and differences of the attached groups. If the two attached groups have different numbers of carbon atoms, they are necessarily totally dissimilar, and this is represented symbolically by  $R_1 \sim_0 R_2$ . The two groups may have the same number of carbon atoms, but if their asymmetry numbers are different they are alike in only this one respect, and this is represented by  $R_1 \sim_1 R_2$ ; if the asymmetry numbers are also the same, the groups may or may not be alike structurally; thus the propyl groups



have the same asymmetry number, 1, but are structurally different. This is represented by  $R_1 \sim_2 R_2$ . If, on the other hand, they are also structurally alike, and the asymmetry number is two or greater, they may still differ from each other enantiomorphically, that is, *d* from *l*; similarity to this extent is represented by  $R_1 \sim_3 R_2$ . The symbol  $R_1 \sim_4 R_2$  to indicate absolute identity in all respects—number of carbon atoms, asymmetry

number, structural arrangement, and stereochemical configuration—would seem natural but turns out to be unnecessary.

Where three groups are attached to the carbon atom bearing the hydroxyl group,  $R_1 \sim_0 R_2 \sim_0 R_3$  indicates that the three attached groups are all of unequal carbon content;  $R_1 \sim_1 R_2 \sim_0 R_3$  that two of the groups are of equal carbon content but of different asymmetry number;  $R_1 \sim_2 R_2 \sim_1 R_3$  that all have the same carbon content and that two groups also have the same asymmetry number and are structurally alike but that the third group has a different asymmetry number; and so on. The first of these statements as given would make a greater similarity between  $R_1$  and  $R_3$  possible. We shall, however, adopt the convention that radicals connected by such similarity chains have the lowest degree of similarity mentioned in any intermediate link.

In the case of the secondary alcohols, the sum of the carbon atoms in the two attached groups is one less than the total number of carbon atoms. Two cases arise: (a) if  $n$  is an even number, then the groups cannot be of equal carbon content, and (b) if  $n$  is odd, they may. For either case a contribution to the number of secondary alcohols,  $s_{n,a}$ , is given by

$$\text{Type 2} \quad \sum_{\substack{i,j \\ i+j=n-1 \\ i>j}} T_{i,\beta} T_{j,\gamma} \quad R_1 \sim_0 R_2$$

$$\alpha = 2\beta\gamma$$

where  $i$  is the number of carbon atoms in one of the attached groups,  $j$  the number of carbon atoms in the other, and  $\beta$  and  $\gamma$  are the asymmetry numbers of the groups. A new center of asymmetry has been created in this process, since all of the groups attached to the central carbon atoms are different, and the asymmetry number is two times the product of the asymmetry numbers of the two groups.

In case (b),  $n$  odd, there are in addition the further combinations in which the two groups have the same number of carbon atoms. This gives rise to three types

$$\text{Type 3} \quad \sum_{\alpha=2\beta\gamma} T_{\frac{n-1}{2},\beta} T_{\frac{n-1}{2},\gamma} \quad R_1 \sim_1 R_2$$

$$\text{Type 4} \quad \frac{1}{2} T_{\frac{n-1}{2},\beta} (T_{\frac{n-1}{2},\beta} - 1) \quad R_1 \sim_2 R_2$$

$$\alpha = 2\beta^2$$

$$\text{Type 5} \quad \alpha = 2\beta^3 \quad T_{\frac{n-1}{2},\beta} \quad R_1 \sim_3 R_2$$

$s_{n,a}$  is then the sum of the numbers found for these four types.

The formula for type 3, the first of these three formulas, is immediately obvious, since for each of the groups  $T_{\frac{n-1}{2},\beta}$  there can be attached to

the carbon atom carrying the  $-\text{OH}$  group  $T_{\frac{n-1}{2},\gamma}$  groups, and since the

groups are of different asymmetry number and different structurally a new asymmetric carbon atom has been created, and therefore  $\alpha = 2\beta\gamma$ . Type 4 deals with the case where the two groups have the same asymmetry number but may still be structurally different; the total number is given by<sup>8</sup>

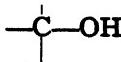
$$T_{\frac{n-1}{2}, \beta}^{C_2}$$

This is the number of distinct pairs of the  $T_{\frac{n-1}{2}, \beta}$  isomers. The factor  $\frac{1}{2}$

enters since the two positions are identical. A new asymmetric carbon atom appears here and so  $\alpha = 2\beta^2$ . Type 5 deals with the case where the two groups have the same asymmetry number, are alike structurally, but differ enantiomorphically. There are  $T_{\frac{n-1}{2}, \beta}$  such groups, and for any one of

these,  $\beta$  may occupy one position and  $\beta$  the other, the new asymmetry number being  $\alpha = \beta^2$ . Of these, in  $\beta(\beta - 1)$  cases, the radicals are different enantiomorphically. Interesting members of this case are the compounds containing three asymmetric carbon atoms two of which are identical. The first alcohol of this type is the nine-carbon alcohol di-sec-butyl carbinol, each of the butyl radicals having an asymmetric carbon atom,  $\beta$  thus being 2. Either a *d*- or *l*-sec-butyl group may occupy one position and either a *d*- or *l*-sec-butyl the second. The combinations *d*-sec-butyl-*l*-sec-butyl and *l*-sec-butyl-*d*-sec-butyl are enantiomeric, a new asymmetric carbon atom having been created since the groups are not alike. The central carbon atom does not become asymmetric in the combinations *d*-sec-butyl-*d*-sec-butyl or *l*-sec-butyl-*l*-sec-butyl since the two groups are identical in each case. Four stereoisomeric forms thus exist as indicated by the value of  $\alpha = 4$ .

The tertiary alcohols (types 6-15) may be derived from the carbon atom carrying the hydroxyl group



by the attachment of three alkyl groups,  $R_1$ ,  $R_2$ , and  $R_3$  such that the sum of the numbers of carbon atoms in the three groups is  $n - 1$ . Three cases arise, depending on whether the carbon contents of the three groups are (a) all different (type 6), (b) two the same (types 7 through 9), or (c) all the same (types 7 through 15).

For case (a) the number of tertiary alcohols is given by

$$\text{Type 6} \quad \sum_{\substack{i,j,k \\ i+j+k=n-1 \\ i>j>k}} \beta, \gamma, \delta \quad T_{i,\beta} T_{j,\gamma} T_{k,\delta} \quad R_1 \sim_0 R_2 \sim_0 R_3$$

$i$ ,  $j$ , and  $k$  being the carbon contents of the attached groups, and  $\beta$ ,  $\gamma$ , and

$${}^n C_r = \frac{n(n-1)(n-2)\dots(n-r+1)}{r!}, \text{ the number of combinations of } n \text{ objects taken } r \text{ at a time.}$$

δ their asymmetry numbers. The asymmetry number of these alcohols is twice the product of the asymmetry numbers of the groups R<sub>1</sub>, R<sub>2</sub>, and R<sub>3</sub>, since a new asymmetric carbon atom is created.

Case (b) in which two of the groups are of equal carbon content is quite similar to case (b) of the secondary alcohols where the two attached groups are of equal carbon content, the number of isomeric forms of the unlike group T<sub>i,γ</sub>, entering as a multiplying factor.

Type 7	$\sum_{i,j}$ $i \neq j$ $2i + j = n - 1$	$\sum_{\beta,\gamma,\delta}$ $\beta > \gamma$ $a = 2\beta\gamma\delta$	T <sub>i,β</sub> T <sub>i,γ</sub> T <sub>i,δ</sub>	R <sub>1~1</sub> R <sub>2~0</sub> R <sub>3</sub>
Type 8	$\frac{1}{2} \sum_{i,j}$	$\sum_{\beta,\gamma}$ $a = 2\beta^2\gamma$	T <sub>i,β</sub> (T <sub>i,β</sub> - 1) T <sub>i,γ</sub>	R <sub>1~2</sub> R <sub>2~0</sub> R <sub>3</sub>
Type 9	$\sum_{i,j}$	$\sum_{\beta,\gamma}$ $a = \beta^2\gamma$	T <sub>i,β</sub> T <sub>i,γ</sub>	R <sub>1~3</sub> R <sub>2~0</sub> R <sub>3</sub>

Case (c) in which all three groups are of the same carbon content can only occur when the number of carbon atoms is one greater than a multiple of three.

Type 10	$\sum_{\beta,\gamma,\delta}$ $3i = n - 1$ $\beta > \gamma > \delta$ $a = 2\beta\gamma\delta$	T <sub>i,β</sub> T <sub>i,γ</sub> T <sub>i,δ</sub>	R <sub>1~1</sub> R <sub>2~1</sub> R <sub>3</sub>
Type 11	$\frac{1}{2} \sum_{\beta,\gamma}$ $\beta \neq \gamma$ $a = 2\beta^2\gamma$	T <sub>i,β</sub> (T <sub>i,β</sub> - 1) T <sub>i,γ</sub>	R <sub>1~2</sub> R <sub>2~1</sub> R <sub>3</sub>
Type 12	$\sum_{\beta,\gamma}$ $\beta \neq \gamma$ $a = \beta^2\gamma$	T <sub>i,β</sub> T <sub>i,γ</sub>	R <sub>1~3</sub> R <sub>2~1</sub> R <sub>3</sub>
Type 13	$a = 2\beta^2$	$1/6 T_{i,\beta} (T_{i,\beta} - 1) (T_{i,\beta} - 2)$	R <sub>1~2</sub> R <sub>2~2</sub> R <sub>3</sub>
Type 14	$a = \beta^2$	T <sub>i,β</sub> (T <sub>i,β</sub> - 1)	R <sub>1~3</sub> R <sub>2~2</sub> R <sub>3</sub>
Type 15	$a = (\beta/3)(\beta^2 + 2)$	T <sub>i,β</sub>	R <sub>1~3</sub> R <sub>2~3</sub> R <sub>3</sub>

In type 10 the three groups are of unequal asymmetry numbers, and the number of new alcohols is simply the product of the numbers of alcohols of the three groups summed over all the possible combinations of groups such that the total carbon content of the three groups is n - 1, and over all values of their asymmetry numbers which occur.

In type 11 two groups have the same asymmetry number but still differ structurally, and the third group has a different asymmetry number; the situation is similar to types 4 and 8. In type 12 two groups are alike structurally, the third having a different asymmetry number; this type is analogous to types 5 and 9.

In type 13 all of the three attached groups have the same asymmetry number but differ structurally. The first position may be occupied by  $T_{i,\beta}$  groups, the second by  $T_{i,\beta} - 1$ , and the third by  $T_{i,\beta} - 2$ ; many of these are the same, however, since the three positions are identical, so that the number of different isomers is given by  $1/6$  of the product (three different groups can occupy three positions six different ways, but, if the positions are identical, in only one way). A new asymmetric carbon atom is created so that the asymmetry number is twice the product of the asymmetry numbers of the groups, that is  $\alpha = 2\beta^3$ .

Two of the attached groups become structurally alike in type 14 so that  $T_{i,\beta}$  forms can occupy the first and second positions,  $T_{i,\beta} - 1$  the third; the number of new isomers then is the product  $T_{i,\beta}(T_{i,\beta} - 1)$ . No new asymmetric carbon is created here so that the asymmetry number is merely the product of the asymmetry numbers of the three groups.

The last type deals with the case where all three of the attached groups are structurally alike, differing only stereoisomerically. There are  $T_{i,\beta}$  such forms, and the asymmetry of the alcohol molecules formed will depend on the enantiomorphic relations of the attached groups. In one case these could all be different, for example, for a group of asymmetry number four, the three could be *dd*, *dl*, and *ld*; a new asymmetric carbon would then be formed, and we have the contribution to  $\alpha$  of  $2\beta C_3^*$ . Or two of the three may be absolutely identical, or again all three may be; in either case no new asymmetric carbon atom is formed, and the asymmetry number then has the further terms  $\beta(\beta - 1)$  and  $\beta$ . Accordingly,

$$\alpha = 2/6 \beta(\beta - 1)(\beta - 2) + \beta(\beta - 1) + \beta = \beta/3(\beta^2 + 2)$$

as given above. An exceptionally interesting compound of this type is the nineteen-carbon alcohol  $[C_2H_5CH(CH_3)CH(CH_3)]_3COH$  which should exist in twenty-four forms, in eight of which the central carbon atom is asymmetric.

The use of these formulas in computing the number of alcohols of a given carbon content is illustrated in the following example, Table I, in which the number of ten-carbon alcohols of the various types is calculated. The values for the lower alcohols needed are taken from Table II.

We then tabulate the values obtained in this manner (Table IB), grouping all of the forms for a particular value of the asymmetry number together, still indicating from which type they were derived and whether primary, secondary, or tertiary:

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\*  $C_r = \frac{n(n-1)(n-2)\dots(n-r+1)}{r!}$ , the number of combinations of  $n$  objects taken  $r$  at a time.

This process has been extended through the twenty-carbon alcohols, and the results are summarized in Table II. For each value of  $n$  and  $\alpha$ , the numbers of primary, secondary, and tertiary alcohols are given, and the total numbers of structural and stereoisomers for the primary, secondary, and tertiary alcohols, and also for each asymmetry number, are given.

The values obtained in this manner for the total number of structural isomers and stereoisomers agree with the values given by Henze and Blair except in the cases of nineteen- and twenty-carbon alcohols where their values for the total number of stereoisomers is 1,000 lower. These differences are due to an error in the calculation of the number of nineteen-carbon alcohols, for a repetition of the calculation using the method of Henze and Blair<sup>1,2</sup> yields the same results as the present method.

#### SUMMARY

A new method of computing the number of stereoisomeric alcohols, following somewhat the lines previously laid down by Henze and Blair, has been devised, which makes possible the calculation of the number of stereoisomeric alcohols, or monosubstituted saturated aliphatic hydrocarbons in general. A new term, the asymmetry number, has been introduced and the field reduced to fifteen types which make possible the calculation of the number of alcohols of all possible configurations. The number of stereoisomeric alcohols of each of the asymmetry numbers which occur through the twenty-carbon alcohols has been computed.

TABLE I  
THE COMPUTATION OF THE NUMBER OF STEREOISOMERIC ALCOHOLS CONTAINING TEN CARBON ATOMS

PART A—Application of Formulas

<i>n</i>	Type	Source	Term	<i>a</i>	<i>n</i>	Type	Source	Term	<i>a</i>
10	1	$T_{0,1}$	39	1	10	6	$T_{0,1} T_{2,1} T_{1,1}$	8	2
		$T_{0,2}$	102	2			$T_{0,2} T_{2,1} T_{1,1}$	8	4
		$T_{0,4}$	63	4			$T_{0,4} T_{2,1} T_{1,1}$	1	8
		$T_{0,8}$	7	8			$T_{0,2} T_{2,1} T_{1,1}$	10	2
		$T_{0,1} T_{1,1}$	23	2			$T_{0,2} T_{2,1} T_{1,1}$	6	4
		$T_{0,2} T_{1,1}$	46	4			$T_{4,1} T_{2,1} T_{2,1}$	6	2
	2	$T_{0,4} T_{1,1}$	19	8		7	$T_{4,2} T_{2,1} T_{2,1}$	2	4
		$T_{0,8} T_{1,1}$	1	16			$T_{4,2} T_{4,1} T_{1,1}$	3	4
		$T_{1,1} T_{2,1}$	14	2			$\frac{1}{2}T_{4,1}(T_{4,1}-1) T_{1,1}$	3	2
		$T_{1,2} T_{2,1}$	20	4		8	$T_{4,1} T_{1,1}$	3	1
		$T_{7,4} T_{2,1}$	5	8			$T_{4,2} T_{1,1}$	1	4
		$T_{6,1} T_{2,1}$	16	2			$T_{2,1} T_{2,1}$	5	1
		$T_{4,2} T_{2,1}$	16	4		9	$T_{2,1} T_{5,2}$	3	2
		$T_{4,4} T_{2,1}$	2	8			$T_{1,1} T_{7,1}$	14	1
		$T_{5,1} T_{4,2}$	15	2			$T_{1,1} T_{7,2}$	20	2
	3	$T_{5,2} T_{4,1}$	9	4	14	10	$T_{1,1} T_{7,4}$	5	4
		$T_{5,1} T_{4,3}$	5	4			$T_{2,1}(T_{2,1}-1)$	2	1
		$T_{5,2} T_{4,2}$	3	8			$T_{2,1}$	2	1

PART B—Rearrangement and Summary

<i>n</i>	<i>a</i>	Type	$p_{n,a}$	$s_{n,a}$	$t_{n,a}$	$T_{n,a}$	$aT_{n,a}$
10	1	1	39				
		9			22		
		14			2		
		15			2	65	65
	2	1	102				
		2		68			
		6			24		
		8			3		
		9			23		
		1	63			220	440
		2		96			
		6			16		
		7			3		
		9			6		
	8	1	7			184	736
		2					
		6					
		2		29			
	16	6			1	37	296
		2				1	16
						507	1,553

TABLE II

## THE NUMBERS OF STRUCTURAL AND STEREOISOMERIC ALCOHOLS

*n*=number of carbon atoms    *p*=primary    *s*=secondary    *t*=tertiary  
*a*=asymmetry number (the number of stereoisomers of each of a given structural isomer)

$T_{n,a}$  = number of structural isomers     $aT_{n,a}$  = number of stereoisomers

<i>n</i>	Values of <i>a</i>						$\Sigma T_{n,a}$	$\Sigma aT_{n,a}$
	1	2	4	8	16	32		
1 <i>p</i>	1						1	1
2 <i>p</i>	1						1	1
3 <i>p</i>	1						1	1
<i>s</i>	1						1	1
<i>T<sub>1,a</sub></i>	2						2	2
<i>aT<sub>1,a</sub></i>	2							
4 <i>p</i>	2						2	2
<i>s</i>		1					1	2
<i>t</i>	1		1				1	1
<i>T<sub>2,a</sub></i>	3		1				4	5
<i>aT<sub>2,a</sub></i>	3		2					
5 <i>p</i>	3		1				4	5
<i>s</i>	1		2				3	5
<i>t</i>	1						1	1
<i>T<sub>3,a</sub></i>	5		3				8	11
<i>aT<sub>3,a</sub></i>	5		6					
6 <i>p</i>	5		3				8	11
<i>s</i>		5					6	14
<i>t</i>	3						3	3
<i>T<sub>4,a</sub></i>	8		8				17	28
<i>aT<sub>4,a</sub></i>	8		16					
7 <i>p</i>	8		8				17	28
<i>s</i>	2		9				15	36
<i>t</i>	4		3				7	10
<i>T<sub>5,a</sub></i>	14		20				39	74
<i>aT<sub>5,a</sub></i>	14		40					
8 <i>p</i>	14		20				39	74
<i>s</i>		19	13				33	98
<i>t</i>	9		7				17	27
<i>T<sub>6,a</sub></i>	23		46				89	199
<i>aT<sub>6,a</sub></i>	23		92					
9 <i>p</i>	23		46				89	199
<i>s</i>	3		35				82	273
<i>t</i>	13		21				40	79
<i>T<sub>7,a</sub></i>	39		102				211	551
<i>aT<sub>7,a</sub></i>	39		204					
10 <i>p</i>	39		102				211	551
<i>s</i>		68	96				194	768
<i>t</i>	26		50				102	234
<i>T<sub>8,a</sub></i>	65		220				507	
<i>aT<sub>8,a</sub></i>	65		440					1,553
11 <i>p</i>	65		220				507	1,553
<i>s</i>	5		124				482	2,197
<i>t</i>	40		117				249	686
<i>T<sub>9,a</sub></i>	110		461				1,238	
<i>aT<sub>9,a</sub></i>	110		922	2,012	1,232	160		4,436

TABLE II (Continued)

<i>n</i>	Values of $\alpha$					
	1	2	4	8	16	24
12 <i>p</i>	110	461	503	154	10	
<i>s</i>		232	552	349	54	
<i>t</i>	74	255	245	55	2	
$T_{12,a}$	184	948	1,300	558	66	
$aT_{12,a}$	184	1,896	5,200	4,464	1,056	
13 <i>p</i>	184	948	1,300	558	66	
<i>s</i>	8	420	1,252	1,037	251	
<i>t</i>	118	553	678	234	19	
$T_{13,a}$	310	1,921	3,230	1,829	336	
$aT_{13,a}$	310	3,842	12,920	14,632	5,376	
14 <i>p</i>	310	1,921	3,230	1,829	336	
<i>s</i>		768	2,765	2,909	992	
<i>t</i>	210	1,147	1,754	842	119	
$T_{14,a}$	520	3,836	7,749	5,580	1,447	
$aT_{14,a}$	520	7,672	30,996	44,640	23,152	
15 <i>p</i>	520	3,836	7,749	5,580	1,447	
<i>s</i>	14	1,379	5,995	7,732	3,525	
<i>t</i>	342	2,359	4,363	2,765	585	
$T_{15,a}$	876	7,574	18,107	16,077	5,557	
$aT_{15,a}$	876	15,148	72,428	128,616	88,912	
16 <i>p</i>	876	7,574	18,107	16,077	5,557	
<i>s</i>		2,487	12,673	19,789	11,513	
<i>t</i>	595	4,749	10,552	8,383	2,480	
$T_{16,a}$	1,471	14,810	41,332	44,249	19,550	
$aT_{16,a}$	1,471	29,620	165,328	353,992	312,800	
17 <i>p</i>	1,471	14,810	41,332	44,249	19,550	
<i>s</i>	23	4,437	26,507	48,948	35,340	
<i>t</i>	981	9,458	24,688	24,113	9,349	
$T_{17,a}$	2,475	28,705	92,527	117,310	64,239	
$aT_{17,a}$	2,475	57,410	370,108	928,480	1,027,824	
18 <i>p</i>	2,475	28,705	92,527	117,310	64,239	
<i>s</i>		7,924	54,509	117,897	102,944	
<i>t</i>	1,684	18,583	56,573	66,138	32,483	
$T_{18,a}$	4,159	55,212	203,609	301,345	199,666	
$aT_{18,a}$	4,159	110,424	814,436	2,410,760	3,194,656	
19 <i>p</i>	4,159	55,212	203,609	301,345	199,666	
<i>s</i>	39	14,056	111,066	277,244	287,692	
<i>t</i>	2,798	36,217	126,813	174,902	105,488	1
$T_{19,a}$	6,996	105,485	441,488	753,491	592,846	1
$aT_{19,a}$	6,996	210,970	1,765,952	6,027,928	9,485,536	24
20 <i>p</i>	6,996	105,485	441,488	753,491	592,846	1
<i>s</i>		24,925	223,548	639,122	775,658	
<i>t</i>	4,763	69,890	279,613	448,049	324,840	
$T_{20,a}$	11,759	200,300	944,649	1,840,662	1,693,344	
$aT_{20,a}$	11,759	400,600	3,778,596	14,725,296	27,093,504	24

Values of $\alpha$							
32	64	128	256	512	$\Sigma T_n \alpha$	$\Sigma \alpha T_n$	
1					1,238 1,188 631 3,057	4,436 6,360 2,036 12,832	
1							
32							
1					3,057	12,832	
12					2,980	18,532	
13					1,602	6,112	
416					7,639	37,496	
13					7,639	37,496	
93	1				7,528	54,780	
2					4,074	18,224	
108	1				19,241		
3,456	64					110,500	
108	1				19,241	110,500	
520	16				19,181	162,672	
29					10,443	54,920	
657	17				48,865		
21,024	1,088					328,092	
657	17				48,865	328,092	
2,449	148	1			49,060	486,154	
219	3				26,981	166,245	
3,325	168	1			124,906		
106,400	10,752	128				980,491	
3,325	168	1			124,906	980,491	
10,104	990	20			126,369	1,461,197	
1,291	43				69,923	505,201	
14,720	1,201	21			321,198		
471,040	76,864	2,688				2,946,889	
14,720	1,201	21			321,198	2,946,889	
37,952	5,411	225	1		326,863	4,413,988	
6,305	389	3			182,158	1,541,014	
58,977	7,001	249	1		830,219		
1,887,264	448,064	31,872	256			8,901,891	
58,977	7,001	249	1		830,219	8,901,891	
132,126	25,648	1,754	25		849,650	13,393,855	
27,247	2,614	61			476,141	4,716,540	
218,350	35,263	2,064	26		2,156,010		
6,987,200	2,256,832	264,192	6,656			27,012,286	
218,350	35,263	2,064	26		2,156,010	27,012,286	
433,139	109,111	11,031	327	1	2,216,862	40,807,290	
106,839	14,603	636	4		1,249,237	14,480,699	
758,328	158,977	13,731	357	1	5,622,109		
24,266,496	10,174,528	1,757,568	91,392	512		82,300,275	



# PROPERTIES OF A GENERALIZED OPERATOR

FRED ROBERTSON

From the Department of Mathematics, Iowa State College

## 1. HISTORY AND DEFINITION

Leibnitz, who in 1695 considered the meaning to be given the symbol

$\frac{d^{\frac{1}{2}}y}{dx^{\frac{1}{2}}}$ , was the first to seek a generalized definition of a derivative, but

Liouville is considered the creator of the theory. He used the form

$$(1.1) \quad f(x) = \sum A e^{ax}$$

of which the  $n$ th derivative is  $\Sigma A a^n e^{ax}$  where  $A, a, n$  are any numbers whatsoever, independent of  $x$ .

In 1874, Letnikoff defined the derivative of  $f(x)$  of order  $-p$ , as the value of the integral

$$(1.2) \quad \frac{1}{\Gamma(p)} \int_{x_0}^x f(t) (x-t)^{p-1} dt$$

where  $p$  is a positive number. This definition reduces to that of Liouville and has the advantage that it gives precise results.

Laurent<sup>1</sup> proposed a definition of the generalized derivative which reduced to that of Letnikoff. He chose the value of the integral

$$(1.3) \quad \frac{\Gamma(n+1)}{2\pi i} \int \frac{f(t)}{(t-x)^{n+1}} dt$$

taken along a loop consisting of a rectilinear part and a circular part described about  $x$  as center.

If the function  $f(x)$  may be expanded in a series arranged in ascending powers of  $t-x$ , namely,

$$f(x) = f(t) + (t-x)f'(t) + \frac{(t-x)^2}{2!}f''(t) + \dots,$$

the integral (1.3) becomes

$$\frac{\Gamma(n+1)}{2\pi i} \int \frac{e^{(t-x)s}}{(t-x)^{n+1}} dt \rightarrow f(x)$$

where the symbol  $s$  means  $d/dx$ . The operational expression

$$(1.4) \quad \frac{\Gamma(n+1)}{2\pi i} \int_s \frac{e^{(t-x)s}}{(t-x)^{n+1}} dt$$

taken along the previously designated path, will be called the generalized operator and designated by the symbol  $Q_n(x, s)$ . If  $n$  is a positive integer,

<sup>1</sup>Sur le calcul des dérivées à indices quelconques. Annales des Mathématiques, 1884. 3, série 3, p. 240-53.

a negative integer, or zero, this function is the ordinary  $n$ th derivative,  $n$ th integral or unit operator respectively.

As an illustration let us choose  $n$  positive and less than one; then the value of  $Q_n(x, z)$  along the first part of the loop is

$$\frac{\Gamma(n+1)}{2\pi i} \int_{z_0}^x \frac{e^{(t-x)z}}{(t-x)^{n+1}} dt.$$

Its value along the circular part is zero; and along the last part is

$$\frac{\Gamma(n+1)}{2\pi i} \int_x^{z_0} \frac{e^{(t-x)z}}{(t-x)^{n+1}} dt e^{-2(n+1)\pi i}.$$

The variable  $t$  having turned about the point  $x$ , the exponential factor appears and the derivative is

$$(1.5) \quad \frac{\Gamma(n+1)}{2\pi i} \left( 1 - e^{-2(n+1)\pi i} \right) \int_{z_0}^x \frac{e^{(t-x)z}}{(t-x)^{n+1}} dt;$$

which may be written

$$(1.6) \quad \frac{\Gamma(n+1)}{\pi} \frac{e^{(n+1)\pi i} - e^{-(n+1)\pi i}}{2i} \int_{z_0}^x \frac{e^{(t-x)z}}{(x-t)^{n+1}} dt$$

since

$$e^{(n+1)\pi i} = (e^{\pi i})^{n+1} = (-1)^{n+1}.$$

The derivative (1.5) becomes

$$(1.7) \quad \frac{1}{\Gamma(-n)} \int_{z_0}^x \frac{e^{(t-x)z}}{(x-t)^{n+1}} dt$$

when the relation

$$\frac{\pi}{\sin(n+1)\pi} = \Gamma(-n) \Gamma(n+1)$$

is used.

When  $p$  replaces  $-n$  and the function defined by (1.7) operates upon  $f(x)$ , the result is the definition of Letnikoff.

If we choose  $n = -n'$ ,  $x_0 = 0$ ,  $s = x-t$ , the expression (1.7) becomes

$$(1.8) \quad \frac{1}{\Gamma(n')} \int_0^x e^{-sz} s^{n'-1} ds$$

which is the definition of this operator as given by Davis.<sup>2</sup>

When the number  $n$  is a positive integer one may not neglect the function  $\int \frac{e^{(t-x)z}}{(t-x)^{n+1}} dt$  along the circular part of the path. In this case, the integral along the line contributes nothing; and by virtue of the calculus of residues, that along the circular part may be evaluated.

<sup>2</sup>H. T. Davis. A Survey for Methods of Inversion of Integrals of Volterra Type, Indiana University Studies, No. 76-7, p. 32.

Let us try some examples. Suppose  $n = 1$ ; then

$$\begin{aligned}\frac{\Gamma(n+1)}{2\pi i} \int \frac{e^{(t-x)z}}{(t-x)^{n+1}} dt \rightarrow f(x) &= \frac{\Gamma(2)}{2\pi i} \int \frac{f(t)}{(t-x)^2} dt \\ &= \frac{1}{2\pi i} \frac{d}{dx} \int \frac{f(t)}{(t-x)} dt = f'(x)\end{aligned}$$

since the integral along the line is zero.

Let us suppose  $n = a$ ; then for  $0 < a < 1$  and  $f(t) = a$ , we obtain

$$\begin{aligned}\frac{\Gamma(n+1)}{2\pi i} \int \frac{e^{(t-x)z}}{(t-x)^{n+1}} dt \rightarrow f(x) &= \frac{\Gamma(a+1)}{2\pi i} (1 - e^{-2(a+1)\pi i}) \int \frac{a}{(t-x)^{a+1}} dt \\ &= \frac{1}{\Gamma(-\alpha)} \int_{x_0}^x \frac{a}{(x-t)^{\alpha+1}} dt \\ &= -\frac{a}{\alpha \Gamma(-\alpha)} \frac{d}{dx} \int_{x_0}^x \frac{dt}{(x-t)^\alpha} \\ &= -\frac{a}{\alpha \Gamma(-\alpha) (x-x_0)^\alpha}\end{aligned}$$

which is the  $a$  derivative of the constant  $a$ .

If  $n = -m$  where  $m$  is a positive integer, and  $x_0 = 0$ , then

$$\frac{\Gamma(n+1)}{2\pi i} \int \frac{e^{(t-x)z}}{(t-x)^{n+1}} dt$$

becomes

$$\frac{\Gamma(1-m)}{\pi} \frac{1 - e^{-2(1-m)\pi i}}{2i} \int_0^x e^{(t-x)z} (t-x)^{m-1} dt,$$

or

$$\frac{1}{\Gamma(m)} \int_0^x e^{(t-x)z} (x-t)^{m-1} dt$$

which is

$$(1.9) \quad \int_0^x \cdots \int_0^x e^{(t-x)z} (dt)^m$$

by a theorem of integral equations. The expression (1.9) is the ordinary  $n$ th integral operator of the calculus.

Let us suppose  $x_0 = 0$  and pass to the limit as  $x$  increases without bound for  $n$  positive, or as  $x$  approaches zero for  $n$  negative; then (1.9) becomes

$$(1.10) \quad \lim_{\substack{\text{for } n > 0 \ x \rightarrow \infty \\ \text{for } n < 0 \ x \rightarrow 0}} \int_0^x \cdots \int_0^x e^{(t-x)z} (dt)^n = \frac{1}{z^n}$$

which by the definition of  $z$  is the ordinary  $n$ th integral for  $n$  positive; the ordinary  $n$ th derivative for  $n$  negative. Operationally equations (1.9) and (1.10) are equivalent.

## 2. THE DIFFERENTIAL EQUATION

The operations indicated in the table

$$\begin{array}{c|c} -nx & Q_n(x, z) = \frac{\Gamma(n+1)}{2\pi i} \int \frac{e^{(t-x)z}}{(t-x)^{n+1}} dt \\ 1+xz-n & \frac{\partial}{\partial z} Q_n(x, z) = \frac{\Gamma(n+1)}{2\pi i} \int \frac{e^{(t-x)z}}{(t-x)^n} dt \\ z & \frac{\partial^2}{\partial z^2} Q_n(x, z) = \frac{\Gamma(n+1)}{2\pi i} \int \frac{e^{(t-x)z}}{(t-x)^{n-1}} dt \end{array}$$

lead to the differential equation

$$(2.1) \quad \left( z \frac{\partial^2}{\partial z^2} + (1+xz-n) \frac{\partial}{\partial z} - nx \right) Q_n(x, z) = \frac{\Gamma(n+1)}{2\pi i} \int \frac{e^{(t-x)z}}{(t-x)^{n+1}} \left[ \frac{(n-1)^2}{z} + (1-n)x \right] dt = 0$$

which will be written in the form

$$(2.2) \quad z U_n'''(x, z) + (1+xz-n) U_n'(x, z) - nx U_n(x, z) = 0.$$

A second independent solution of (2.2) is  $z^{-n}$  as may be determined by substitution. These two independent solutions of equation (2.2) are equivalent operators.

The differentiation of each member of equation (2.2) partially with respect to the parameter  $n$  leads to the logarithmic operator.<sup>3</sup>

## 3. THE DIFFERENCE EQUATION

The partial derivatives of the set of functions  $Q_n(x, z)$  with respect to  $z$  may be expressed in terms of functions of the set. For instance

$$(3.1) \quad \frac{\partial Q_n(x, z)}{\partial z} = \frac{n \Gamma(n)}{2\pi i} \int \frac{e^{(t-x)z}}{(t-x)^n} dt = -n Q_{n+1}(x, z).$$

Similarly

$$(3.2) \quad \frac{\partial^2 Q_n(x, z)}{\partial z^2} = n(n+1) Q_{n+2}(x, z).$$

Inserting the results (3.1) and (3.2) in equation (2.1) and lowering the subscripts by unity yield the recursion relation

$$n Q_{n+1}(x, z) - (n+xz) Q_n(x, z) - x Q_{n-1}(x, z) = 0$$

connecting any three consecutive functions of the set.

## 4. A SYMMETRIC RELATION CONNECTING THE FUNCTIONS AND THEIR FIRST PARTIAL DERIVATIVES

The first partial derivatives of  $Q_n(x, z)$  are

$$\frac{\partial Q_n(x, z)}{\partial x} = \frac{x^{n-1} e^{-xz}}{\Gamma(n)}$$

<sup>3</sup> Fred Robertson, "The General Differential Operator," Iowa State College Journal of Science, Vol. XIV, No. 3, 1940, pages 261-66.

and

$$\frac{\partial Q_n(x, z)}{\partial z} = -n Q_{n+1}(x, z).$$

The first partial derivatives with respect to  $x$  was obtained from the expression for the function given by formula (1.8).

Multiplying the first of these partial derivatives by  $x$ , the second by  $z$ , and subtracting gives

$$(4.1) \quad x \frac{\partial Q_n(x, z)}{\partial x} - z \frac{\partial Q_n(x, z)}{\partial z} = \frac{x^n e^{-xz}}{\Gamma(n)} + nx Q_{n+1}(x, z).$$

The result of an integration of  $Q_n(x, z)$  (by parts) expressed as functions of the set gives

$$\begin{aligned} Q_n(x, z) &= \frac{x^n e^{-xz}}{\Gamma(n)} + \int \frac{zs^n e^{-sz}}{\Gamma(n)} ds - (n-1) \int \frac{s^{n-1} e^{-sz}}{\Gamma(n)} ds \\ &= \frac{x^n e^{-xz}}{\Gamma(n)} + nz Q_{n+1}(x, z) - (n-1) Q_n(x, z) \end{aligned}$$

and therefore

$$(4.2) \quad x \frac{\partial Q_n(x, z)}{\partial x} - z \frac{\partial Q_n(x, z)}{\partial z} = n Q_n(x, z).$$

The formula (4.2) is analogous to Euler's formula for homogeneous functions.

## 5. THE INTEGRAL EQUATION

Let us write the partial differential equation (2.1) in the form

$$(5.1) \quad \frac{\partial}{\partial z} \left[ z \frac{\partial U_n(x, z)}{\partial z} \right] + (xz - n) \frac{\partial U_n(x, z)}{\partial z} - nx U_n(x, z) = 0.$$

Multiply each member of the equation (5.1) by  $dz$  and integrate from infinity when  $n$  is a negative number, or from zero when  $n$  is a positive number to  $z$ . An integration by parts results in the partial integro-differential equation

$$(5.2) \quad z \frac{\partial U_n(x, z)}{\partial z} + (xz - n) U_n(x, z) - (n+1)x \int^z U_n(x, s) ds = 0$$

provided  $n \neq -1$ .

The preceding process when applied to the equation (5.2) yields

$$(5.3) \quad z U_n(x, z) + \int^z \left[ (n+2)xs - (n+1)(xz+1) \right] U_n(x, s) ds = 0$$

where for  $n < 0$  the lower limit of the integral is  $\infty$  and for  $n > 0$  it is 0, when one replaces the double integral by an equivalent single integral and provided  $n \neq -1$  or  $-2$ . This equation (5.3) is the integral equation with a parameter which the set of generalized operators satisfies.

## 6. THE GENERATING FUNCTION

Let us define the function  $U(x, z, s)$  by the series

$$(6.1) \quad U(x, z, s) = \sum_{n=1}^{\infty} Q_n(x, z) s^{n-1}.$$

In equation (6.1) the parameter will be restricted to positive integral values. Substituting for  $Q_n(x, z)$  its value from equation (1.8), interchanging the order of integration and adding, transforms the equation (6.1) into

$$(6.2) \quad U(x, z, s) = \frac{1 - e^{-(z-s)x}}{z - s}.$$

The function  $U(x, z, s)$  defined by equation (6.2) is called the generating function of the ordinary integral operators of the calculus. Passing to the limit as  $x$  becomes infinite gives

$$(6.3) \quad U(z, s) = \frac{1}{z - s}.$$

The function defined by (6.3) is called the generating function of the ordinary integral operators of the calculus. It is understood they are to

be expressed in polar form, namely,  $\frac{1}{z^n}$ .

One easily verifies that the function  $U(x, z, s)$  satisfies a homogeneous partial differential equation of parabolic type; namely,

$$\left( \frac{\partial^2}{\partial z^2} + 2 \frac{\partial^2}{\partial x \partial z} + \frac{\partial^2}{\partial x^2} \right) \rightarrow U(x, z, s) = 0.$$

## 7. SOME FORMS OF THE OPERATORS

The generating function  $U(x, z, s)$  may be used to obtain an expression for the functions  $Q_n(x, z)$ . For instance multiplying each member of

equation (6.1) by  $\frac{ds}{2\pi i s^n}$ ; integrating in a counterclockwise direction along

a closed contour surrounding the origin, and using the relation of Pringsheim<sup>4</sup>

$$(7.1) \quad \frac{1}{2\pi i} \int^{0+} t^{m-n-1} dt = \delta_m^n,$$

yields

$$(7.2) \quad Q_n(x, z) = \frac{1}{2\pi i} \int^{0+} \frac{1 - e^{-(z-s)x}}{z - s} \frac{ds}{s^n}.$$

It is to be remembered that  $n$  is restricted to positive integral values. Another useful form of the function is found immediately from the

<sup>4</sup>Pringsheim, Jahrbuch über die Fortschritte der Mathematik, 48 (1921-22) p. 317.

generating function by evaluation of the  $n - 1$  partial derivatives with respect to  $s$  at  $s = 0$ . The result is

$$(7.3) \quad Q_n(x, z) = \frac{1}{(n-1)!} \left. \frac{\partial^{n-1} U(x, z, s)}{\partial s^{n-1}} \right|_{s=0} \\ = \frac{1 - \left( 1 + xz + \frac{x^2 z^2}{2!} + \dots + \frac{x^{n-1} z^{n-1}}{(n-1)!} \right) e^{-zs}}{z^n}.$$

A form of the functions, which will be needed later, is obtained by the substitution  $(t - x) = ux$  in formula (1.4). Then

$$Q_n(x, z) = \frac{\Gamma(n+1)}{2\pi i} \int_{(t-x)^{n+1}} \frac{e^{(t-x)z}}{d(t-x)} dt = x^n \frac{\Gamma(n+1)}{2\pi i} \int \frac{e^{uxz}}{u^{n+1}} du.$$

The two integrals being of the same form the relation

$$(7.4) \quad Q_n(x, z) = x^n Q_n(xz)$$

follows. The expression for the functions in the right hand member of equation (7.4) involves one variable, namely  $xz$ . This form of the functions evidently satisfies the differential equation

$$(7.5) \quad s \frac{d^2 U_n(s)}{ds^2} + (1+s+n) \frac{d U_n(s)}{ds} + n U_n(s) = 0$$

where  $s$  is written for  $xz$ .

The equation (7.5) is called a hypergeometric equation<sup>5</sup> and its solution is represented by the symbol  $F(n, n+1, -s)$ . Its series representation is

$$(7.6) \quad F(n, n+1 - s) = 1 + \frac{n(-s)}{1(n+1)} + \frac{n(n+1)(-s)^2}{1 \cdot 2(n+1)(n+2)} \\ + \frac{n(n+1)(n+2)(-s)^3}{1 \cdot 2 \cdot 3(n+1)(n+2)(n+3)} + \dots$$

The solutions of equation (7.5) may be represented in the following forms for  $n$  a positive integer.

$$\text{a. } U_n(s) = \frac{d^{n-1} \left[ \frac{e^{-s}}{s} \right]}{ds^{n-1}}.$$

$$\text{b. } U_n(s) = \frac{1}{s^n} \int s^{n-1} e^{-s} ds$$

$$\text{c. } U_n(s) = \frac{1}{s^n}.$$

Collecting these forms for the operators, we have

$$Q_n(x, z) = x^n Q_n(s) = x^n n! F(n, n+1, -s) = x^n \sum_{r=0}^{\infty} \frac{(-1)^r x^r z^r}{(n+r) r!} \\ = \frac{1}{s^n} = \frac{1}{s^n} \int s^{n-1} e^{-s} ds$$

where  $s = xz$ .

<sup>5</sup>H. Bateman. Differential Equations. p. 112.

## 8. LINEAR PROPERTY

Let us now show

$$Q_m(x, z) \rightarrow Q_n(x, z) \rightarrow f(x) \equiv Q_{m+n}(x, z) \rightarrow f(x).$$

We recall that

$$Q_n(x, z) = \frac{\Gamma(n+1)}{2\pi i} \int \frac{e^{(t-x)z}}{(t-x)^{n+1}} dt.$$

We may take the  $n$ th derivative by differentiating under the sign of integration; since this differentiation of the operator represents an integration and a multiplication by a constant factor as we see from (3.1). Thus  $Q_m(x, z)$  operating upon the results of  $Q_n(x, z)$  operating upon  $f(x)$  gives

$$\begin{aligned} Q_m(x, z) \rightarrow Q_n(x, z) \rightarrow f(x) &= \frac{\Gamma(n+1)}{2\pi i} \int \frac{e^{(t-x)z}}{(t-x)^{m+n+1}} \frac{\Gamma(m+n+1)}{\Gamma(n+1)} dt \rightarrow f(x) \\ (8.1) \quad &= Q_{m+n}(x, z) \rightarrow f(x). \end{aligned}$$

The rule of Leibnitz applies and

$$\begin{aligned} \frac{\Gamma(n+1)}{2\pi i} \int \frac{e^{(t-x)z}}{(t-x)^{n+1}} dt \rightarrow \psi(x)\phi(x) &= \frac{\Gamma(n+1)}{2\pi i} \int \frac{\psi(t)\phi(t)dt}{(t-x)^{n+1}} \\ &= \frac{\Gamma(n+1)}{2\pi i} \int \frac{\psi(t)}{(t-x)^{n+1}} [\phi(x) + (t-x)\phi'(x) + \dots] dt \\ &= \frac{\Gamma(n+1)}{2\pi i} \int \left[ \frac{\psi(t)\phi(x)}{(t-x)^n} + \frac{\psi(t)\phi'(x)}{(t-x)^n} + \dots \right] dt \\ (8.2) \quad &= \phi(x) \frac{\Gamma(n+1)}{2\pi i} \int \frac{e^{(t-x)z}}{(t-x)^{n+1}} dt + n\phi'(x) \frac{\Gamma(n)}{2\pi i} \int \frac{e^{(t-x)z}}{(t-x)^n} dt + \dots \end{aligned}$$

which is the expanded form of the product function by the formula of Leibnitz.

The rule of Leibnitz permits the generalization of the formula of Vandermonde, known as the binomial for the factorials. The differentiation of each member of the identity

$$x^{a+b} = x^a x^b$$

$n$  times, gives

$$\begin{aligned} \frac{\Gamma(a+b+1)}{\Gamma(a+b-n+1)} &= \frac{\Gamma(a+1)}{\Gamma(a-n+1)} + n \frac{\Gamma(a+1)}{\Gamma(a-n+2)} b \\ (8.3) \quad &+ \frac{n(n-1)}{2!} \frac{\Gamma(a+1)}{\Gamma(a-n+3)} b(b-1) + \dots + \frac{\Gamma(a+1)}{\Gamma(a-2n+1)} b(b-1) \\ &\quad , \dots (b-n+2). \end{aligned}$$

## 9. THE ZEROS OF THE OPERATORS

The location of the zeros of the set of functions  $Q_n(x, z)$  for integral values of the parameter is an intriguing problem.

Let us substitute  $s = tx$  in the functions  $Q_n(x, z)$  as defined by (1.8). Then

$$(9.1) \quad Q_n(x, z) = \int_0^x \frac{s^{n-1} e^{-sz}}{\Gamma(n)} ds = \frac{x^n}{\Gamma(n)} \int_0^1 t^{n-1} e^{-tz} dt$$

which will be considered as a function of the one variable  $xz$ .

Since  $n$  is restricted to positive integral values and  $x \neq 0$ , the zeros of  $Q_n(x, z)$  are the same as those of the function  $F(\mu)$  defined by

$$(9.2) \quad F(\mu) \equiv \int_0^1 t^{n-1} e^{-t\mu} dt$$

where  $\mu = xz$ .

If  $\mu$  is complex of the form  $-x - iy$ , then<sup>6</sup>

$$F(\mu) = \int_0^1 t^{n-1} e^{tx} e^{t\mu} dt.$$

Let us break the interval into two parts by the point  $\tau$ ,  $0 < \tau < 1$  such that

$$(9.3) \quad F(\mu) = \int_0^1 t^{n-1} e^{tx} e^{t\mu} dt = \int_0^\tau t^{n-1} e^{tx} e^{t\mu} dt + \int_\tau^1 t^{n-1} e^{tx} e^{t\mu} dt.$$

An integration by parts yields

$$(9.4) \quad F(\mu) - \int_\tau^1 t^{n-1} e^{tx} e^{t\mu} dt = t^{n-1} e^{tx} \frac{e^{t\mu}}{iy} \Big|_0^\tau - \int_0^\tau \frac{e^{t\mu}}{iy} \frac{d}{dt} (t^{n-1} e^{tx}) \\ = \frac{\tau^{n-1} e^{x\tau} e^{\tau\mu}}{iy} - \int_0^\tau \frac{e^{t\mu}}{iy} \frac{d}{dt} (t^{n-1} e^{tx}).$$

The result of taking absolute values is

$$(9.5) \quad y |F(\mu)| + y \left| \int_\tau^1 t^{n-1} e^{tx} e^{t\mu} dt \right| \geq \int_0^\tau \frac{d}{dt} (t^{n-1} e^{tx}) - \left| \int_0^\tau e^{t\mu} \frac{d}{dt} (t^{n-1} e^{tx}) \right|.$$

Now as  $\tau \rightarrow 1$  the right hand member approaches zero.

Then

$$(9.6) \quad y |F(\mu)| > 0.$$

Thus the real part and the imaginary part of  $F(\mu)$  cannot both vanish at the same time. Then  $F(\mu)$  has no zero for the left half of the plane  $Rz < 0$ .<sup>\*</sup> Polya has given the conditions for which this result is valid.

A more precise result concerning the zeros of this set of functions may be obtained as follows. We recall the exponential form of  $Q_n(x, z)$ , namely,

$$\int_0^x \frac{s^{n-1} e^{-sz}}{\Gamma(n)} ds = x^n \left[ \frac{1 - \left(1 + \mu + \frac{\mu^2}{2!} + \cdots + \frac{\mu^{n-1}}{(n-1)!}\right) e^{-\mu}}{\mu^n} \right]$$

where  $\mu = xz$ .

<sup>6</sup> G. Polya und G. Szegö, Aufgaben und Lehrsätze Aus der Analysis II. p. 170.

\* G. Polya, Math. Zeitschr. Bd. 2, S 355-58, 1918.

The right hand member is zero if

$$e^\mu = 1 + \mu + \frac{\mu^2}{2!} + \cdots + \frac{\mu^{n-1}}{(n-1)!},$$

or

$$(9.7) \quad \frac{1}{n!} + \frac{\mu}{(n+1)!} + \frac{\mu^2}{(n+2)!} + \cdots = 0.$$

Consider the function

$$(9.8) \quad F(\mu) = \frac{\Gamma(n)}{\Gamma(n+1)} + \frac{\Gamma(n)\mu}{\Gamma(n+2)} + \frac{\Gamma(n)\mu^2}{\Gamma(n+3)} + \cdots$$

obtained by multiplying each term of the expression (9.7) by  $\Gamma(n)$ , and replacing the factorials by equivalent Gamma functions. Then  $F(\mu)$  can be written as

$$(9.9) \quad F(\mu) = \sum_{r=0}^{\infty} \frac{\Gamma(r+1) \Gamma(n) \mu^r}{r! \Gamma(n+r+1)}$$

or

$$F(\mu) = \sum_{r=0}^{\infty} \frac{\mu^r}{r!} \int_0^1 t^r (1-t)^{n-1} dt.$$

The latter form is obtained by introducing the Beta function which satisfies the relation

$$\frac{\Gamma(r+1) \Gamma(n)}{\Gamma(n+r+1)} = \int_0^1 t^r (1-t)^{n-1} dt.$$

Changing the order of integration and adding yields

$$F(\mu) = \int_0^1 e^{\mu t} (1-t)^{n-1} dt$$

or

$$(9.10) \quad F(\mu) = \int_0^1 e^{[\mu - (n-1)]t} \left[ e^t (1-t) \right]^{n-1} dt.$$

According to the results obtained by Polya<sup>7</sup> this function for

$$\begin{aligned} n > 1 &\text{ has zeros in } Rz > n-1 \\ 0 < n < 1 &\text{ has zeros in } Rz < n-1 \\ n = 1 &\text{ has zeros on line } Rz = 0. \end{aligned}$$

When

$$\begin{aligned} n = 1 &\text{ the function } F(\mu) \text{ is} \\ e^\mu &= 1. \end{aligned}$$

On separating the real and imaginary parts we have

$$\begin{aligned} e^\xi \cos \eta &= 1, \\ e^\xi \sin \eta &= 0. \end{aligned}$$

<sup>7</sup>G. Polya, Math. Zeitschr. Bd. 2, S 355-58, 1918.

which has solutions

$$\xi = 0, \eta = 2n\pi i.$$

Thus for  $n = 1$  we have shown the zeros of the function  $Q_1(\mu)$  are  $0 + 2n\pi i$  and lie on the line  $Rz = 0$  which agrees with the result obtained by Polya.

#### 10. RELATIONS BETWEEN POLAR AND GENERATRIX OPERATORS

Let us consider equivalent operative properties of linearly independent solutions of the partial differential equation (2.2) for integral values of  $n$ . Symbolically

$$\frac{1}{z^n} \rightarrow f(x) \equiv Q_n(x, z) \rightarrow f(x).$$

The operator  $\frac{1}{z^n}$  will be called polar and  $Q_n(x, z)$  the equivalent generatrix operator.

We shall derive an expression for the generatrix operator involving the generatrix function  $u(x, z, t)$ . The multiplication of each member of

equation (6.1) by  $\frac{t^m}{2\pi i}$  and the integration in a counterclockwise direction

along a closed contour surrounding the origin, give

$$(10.1) \quad \frac{1}{2\pi i} \int^{0+} \frac{u(x, z, t)}{t^m} dt = \sum_{n=1}^{\infty} \frac{Q_n(x, z)}{2\pi i} \int^{0+} t^{m-n-1} dt.$$

For the values of  $n$  which we have chosen, the path of integration may be any circle surrounding the origin.

The relation of Pringsheim<sup>8</sup>

$$\frac{1}{2\pi i} \int^{0+} t^{m-n-1} dt = \delta_m^n$$

applied to the right hand member of equation (10.1) yields

$$Q_n(x, z) = \frac{1}{2\pi i} \int^{0+} \frac{u(x, z, t)}{t^n} dt$$

which by (6.2) becomes

$$(10.2) \quad Q_n(x, z) = \frac{1}{2\pi i} \int^{0+} \frac{1 - e^{-(z-t)x}}{z-t} \frac{dt}{t^n}.$$

The function  $\frac{1}{z^n}$  has a simple polar singularity at  $z = 0$  or order  $n$ ,

but its corresponding generatrix function is entire in  $z$ . Functions of

<sup>8</sup>Pringsheim, Jahrbuch über die Fortschritte der Mathematik, 48 (1921-22) p. 317.

operators must satisfy more stringent conditions if they are generatrix functions than if they are equivalent polar operative functions.

Theorem: If  $f(z)$  is a polar operative function with a simple polar singularity at  $z = a$  then its corresponding generatrix expression is entire in  $z$ .

The conditions of the theorem require  $f(z)$  to be of the form  $\frac{\phi(z)}{z-a}$

where  $\phi(z)$  is a transcendental integral function of  $z$ . A function of  $z$  which has no singularity in the finite plane and an essential singularity at infinity will be called a transcendental integral function of  $z$ .

Let us recall (10.2), then

$$\begin{aligned} f(z) &= \frac{1}{2\pi i} \int^{0+} \frac{\phi(t)}{t-a} u(x, z, t) dt \\ &= \frac{1}{2\pi i} \int^{0+} \frac{\phi(t)}{t-a} \frac{1 - e^{-(z-t)x}}{z-t} dt \\ &= \phi(a) \frac{1 - e^{-(x-a)x}}{z-a} \end{aligned}$$

As an illustration let  $f(z) = \frac{1}{z} + \frac{1}{z^2} + \dots + \frac{1}{z-1}$ ; then  $a = 1$  and

$$\phi(a) = 1.$$

Therefore<sup>9</sup>

$$\frac{1}{z} + \frac{1}{z^2} + \frac{1}{z^3} + \dots = \frac{e^x e^{-xz} - 1}{1-z}.$$

### 11. FORMAL EXPANSION OF AN ARBITRARY OPERATOR FUNCTION

Before proceeding to the expansion of an arbitrary operator function in terms of a normalized, orthogonal set of functions, we shall develop another general method of expansion.

If  $u(t)$  and  $f(t)$  are two functions of  $t$  for which the necessary integrals and derivatives exist, then

$$\begin{aligned} \int_0^x u(t) f(t) dt &= u(t) \int f(t) dt \Big|_0^x - \int_0^x \int f(t) dt \frac{\partial u}{\partial t} dt \\ &= u(x) \int_0^x f(t) dt + [u(x) - u(0)] \int_0^0 f(t) dt \\ &\quad - \int_0^x \int_0^t f(t) dt \frac{\partial u}{\partial t} dt + \int_0^x f(t) dt \int_0^x \frac{\partial u}{\partial t} dt \\ (11.1) \qquad \qquad \qquad &= u(t) \Big|_{t=x} \int_0^x f(t) dt - \int_0^x \left[ \int_0^t f(t) dt \frac{\partial u}{\partial t} \right] dt. \end{aligned}$$

In the development of formula (11.1) an integral with but one limit means the indefinite integral is to be evaluated at that limit only.

<sup>9</sup>H. T. Davis, Theory of Volterra Integral Equations of Second Kind. Indiana University Studies No's 88-89-90. p. 34.

Repeated application of formula (11.1) gives

$$\begin{aligned}
 \int_0^x u(t) f(t) dt &= u(t) \Big|_{t=x} \int_0^x f(t) dt - \frac{\partial u}{\partial t} \Big|_{t=x} \int_0^x \int_0^{t_1} f(t) dt dt_1 \\
 &\quad + \frac{\partial^2 u}{\partial t^2} \Big|_{t=x} \int_0^x \int_0^{t_1} \int_0^{t_2} f(t) \cdots dt_2 dt_1 \\
 (11.2) \quad &= \sum_{n=0}^{\infty} (-1)^n \frac{\partial^n u(t)}{\partial t^n} \Big|_{t=x} \int_0^x f(t) dt^n.
 \end{aligned}$$

The operational analogue of formula (11.2), namely,

$$(11.3) \quad \int_0^x u(t) e^{(t-x)z} dt = \sum_{n=0}^{\infty} (-1)^n \frac{\partial^n u(t)}{\partial t^n} \Big|_{t=x} Q_{n+1}(x, z)$$

is obtained by choosing  $f(t) = e^{(t-x)z}$ .<sup>10</sup>

Formula (11.3) is a special case of the expansion obtained when the substitution

$$f(t) = \int_0^x \frac{(t-x)^{m-1} e^{-(t-x)z}}{\Gamma(m)} dt$$

is made in formula (11.2). The result is

$$(11.4) \quad \int_0^x u(t) Q_m(x, z, t) dt = \sum_{n=0}^{\infty} (-1)^n \frac{\partial^n u(t)}{\partial t^n} \Big|_{t=x} Q_{n+m+1}(x, z).$$

The expansion of an arbitrary operative function in terms of the operator  $\frac{1}{z^n}$  is identical with the well-known expansion for the reciprocals of the simplest polynomials.

To develop an arbitrary operative function in terms of the operators  $Q_n(x, z)$  for integral values of  $n$ , we use the formula of Schmidt\* for the

$$(11.5) \quad \phi_n(x, z) = \frac{Q_n(x, z) - \sum_{k=1}^{n-1} \phi_k(x, z) \int_0^{\infty} \phi_k(x, s) Q_n(x, s) ds}{\sqrt{N\phi_n(x, s)}}$$

discovery of normalized orthogonal systems of functions by linear combinations of a given infinite set defined over a given range, and rearrange the resulting series in terms of the given set of generatrix functions. The symbol  $N\phi_n(x, s)$  is read the norm of  $\phi_n(x, s)$  and is defined by the relation

$$N\phi_n(x, s) = \int_0^{\infty} \phi_n^2(x, s) ds.$$

<sup>10</sup> The formula (11.3) is due to Prof. H. T. Davis of Northwestern University.

\* E. Schmidt, Entwicklung Willkürlichen Funktionen. Mathematische Annalen Vol. 63 (1907) p. 442.

The range of definition of  $x$  shall be the closed interval from zero to  $b$ .

Formulas for the computation of the right hand member of equation (11.1) when the generatrix functions are used, will be developed.

Theorem I. The  $r$ th partial derivative of  $Q_n(x, z)$  with respect to  $z$  expressed in terms of generatrix functions is

$$(11.6) \quad \frac{\partial^r Q_n(x, z)}{\partial z^r} = (-1)^r \frac{(n+r-1)!}{(n-1)!} Q_{n-r}(x, z).$$

The proof follows immediately from (3.1).<sup>11</sup>

Theorem II. The multiplication of  $Q_{n+1}(x, s)$  by  $ds$  and integration between the limits zero to infinity gives

$$(11.7) \quad \int_0^\infty Q_{n+1}(x, s) ds = \frac{x^n}{n!n}$$

provided  $n \neq 0$ .

The proof comes at once from (3.1).

Theorem III. The multiplication of  $Q_n(x, s) Q_k(x, s)$  by  $ds$  and integration between the limits zero to infinity gives

$$(11.8) \quad \int_0^\infty Q_n(x, s) Q_k(x, s) ds = \sum_{r=1}^{k-1} \frac{(-1)^{r-1} x^{n+k-1}}{(k-r)(k-1)!(n-1)!(n+r-1)} \\ + \frac{(-1)^k (n+k-2)!}{(n-1)!(k-1)!} \int_0^\infty Q_1(x, s) Q_{n+k-1}(x, s) ds.$$

The proof follows by integration by parts.

Cor. I. If  $n = k$  formula (11.4) becomes

$$\int_0^\infty Q_k(x, s) Q_k(x, s) ds = \sum_{r=1}^{k-1} \frac{x^{2k-1} (-1)^{r-1}}{(k-r)[(k-1)!]^2 (k+r-1)} \\ + \frac{(-1)^{k-1} (2k-2)!}{[(k-1)!]^2} \int_0^\infty Q_1(x, s) Q_{2k-1}(x, s) ds.$$

Cor. II. If  $n = 1$  formula (11.4) becomes

$$\int_0^\infty Q_1(x, s) Q_k(x, s) ds = \frac{1}{2} \sum_{r=1}^{k-1} \frac{(-1)^{r-1} x^k}{r(k-r)(k-1)!}$$

provided  $k$  is an even integer.

Theorem IV. The multiplication of  $Q_n(x, s) Q_{n+1}(x, s)$  by  $ds$  and an integration between the limits zero to infinity gives

$$(11.9) \quad \int_0^\infty Q_n^k(x, s) Q_{n+1}(x, s) ds = -\frac{1}{n(1+k)} \left[ \frac{x^n}{n!} \right]^{k+1}.$$

The proof comes through an integration by parts.

<sup>11</sup> F. Schuh, Christian Huygens, Vol. 1 (1921-22) p. 82-3.

**Theorem V.** *The multiplication of  $Q_1^2(x, s)$  by  $ds$  and an integration between the limits zero to infinity gives*

$$(11.10) \quad \int_0^\infty Q_1^2(x, s) ds = x^2 \log 2.$$

$$\begin{aligned} \text{Proof: } \frac{\partial}{\partial x} \int_0^\infty Q_1^2(x, s) ds &= 2x \int_0^\infty \frac{e^{-s} - e^{-2s}}{s} ds \\ &= 2x \left[ \int_0^c \frac{e^{-s} - e^{-2s}}{s} ds + \int_c^\infty \frac{e^{-s}}{s} ds - \int_{2c}^\infty \frac{e^{-s}}{s} ds \right] \\ &= 2x \lim_{c \rightarrow 0} \left[ \int_0^c \frac{e^{-s} - e^{-2s}}{s} ds + e^{-z} \int_c^{2c} \frac{ds}{s} \right] \\ &= 2x \log 2. \end{aligned}$$

**Theorem VI.** *The multiplication of  $Q_n^2(x, s)$  by  $ds$  and an integration between the limits zero to infinity gives*

$$(11.11) \quad \int_0^\infty Q_n^2(x, s) ds = \frac{2}{(n-1)!} \left[ \frac{x^{2n-1}}{2n-1} \left\{ \frac{(-1)^n}{(n-1)!(n-1)} \left[ \frac{1}{(n-1)!(n-1)} \right. \right. \right. \\ \left. \left. \left. - \frac{1}{(n-1)!2!} - \frac{2^{n-1}}{(n-1)!2!} + \cdots + \frac{(-1)^{n+1}}{(n-1)!} \log 2 \right] \right\} \right].$$

The proof is similar to that used in the preceding theorem.

The theorems previously stated are sufficient to compute the system of functions  $\phi_i(x, z)$  which form a normalized, orthogonal system of functions

The set  $\phi_i(x, z)$  has the property

$$\int_0^\infty \phi_i(x, s) \phi_j(x, s) ds = \delta_i^j$$

where  $\delta$  is the Kronecker delta. Thus an arbitrary operative function of  $x$  and  $z$  may be formally expanded in a series of the form

$$(11.12) \quad f(x, z) = \sum_{n=1}^{\infty} a_n(x) \phi_n(x, z)$$

where

$$a_n(x) = \int_0^\infty f(x, s) \phi_n(x, s) ds.$$

From this result we write

$$(11.13) \quad f(x, z) = \sum_{n=1}^{\infty} a_n(x) \sum_{i=1}^{\infty} \sum_{\alpha} (-1)^{\beta+i+1} \frac{1}{N_{n \cdots \beta \cdots i}} Q_i(x, z)$$

where  $\alpha$  is every possible combination of  $(n-1) \dots (i-1)$  in the order given,  $\beta$  is the number of indices omitted,  $(\beta = 0, 1) \dots (n-i-1)$ , and

$\frac{1}{N_i}$  is the reciprocal of the square root of the norm if the index is not repeated, but is the product of these expressions multiplied by

$\int_0^\infty \phi_i(x, s) \phi_n(x, s) ds$  when the index is repeated.

Thus the function  $f(x, z)$  has been formally expanded in a series of the form

$$(11.14) \quad f(x, z) = \sum_{n=1}^{\infty} a_n(x) Q_n(x, z)$$

where the  $a_n(x)$  are infinite series.

Conditions on  $f(x, z)$  for which the expansion (11.12) exists and represents the function are given by the Riesz-Fischer<sup>12</sup> and related theorems. Conditions under which the terms of a double series may be rearranged in a specified order and the resulting series represent the function are given by the Weierstrass' theorem on double series.<sup>13</sup>

The equation (11.13) expresses the formal expansion of the operator function  $f(x, z)$  in the form

$$(11.15) \quad f(x, z) = \sum_{n=1}^{\infty} a_n(x) Q_n(x, z)$$

where the coefficients  $a_n(x)$  are infinite series.

<sup>12</sup>E. Fischer, F. Riesz. Comptes Rendus, 144, (1907), 615-619, 734-736.

<sup>13</sup>Konrad Knopf, Theory and Application of Infinite Series. (1928) p. 430.

# HISTOGENESIS IN THE ROOTS OF HOLCUS SORGHUM L.

Hsru-Hui CHI<sup>1</sup>

From the Department of Botany, Iowa State College

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*Holcus sorghum* has long been an important crop in many parts of the world. There have been, however, comparatively few investigations dealing with the morphology of sorghum. The literature on the morphology of other gramineous crop plants is very extensive. Sargent and Arber (9) studied grass embryos and seedlings from the standpoint of comparative morphology and anatomy. Avery (3) compared the homologies of the organs in the embryos of maize, oats and wheat. Percival (7) studied wheat from the developmental viewpoint, and discussed the entire life history of the plant. Martin and Hershey (5) studied the developmental cycle of corn and recognized three periods in the life cycle of the plant. Artschwager (1, 2) described the development and structure of the vegetative organs of sugarcane.

The structure of the embryo and vascular system of the seedling of sorghum has been studied by Reznik (8). The number and arrangement of its temporary roots has been noted by Sieglinger (10); Millar (6) studied the root system and leaf areas, and the superficial rooting habit has been pointed out by Weaver (11). Miss Chen (4) described the histological development of the vascular tissues of the stem of sorghum. The principal aim of the present study was to investigate the morphology, and more specifically the histological development of the root system of sorghum.

## MATERIALS AND METHODS

The sorghum used in this study was the Chinese *Kaoliang* variety. The seed was obtained from the Seed Laboratory of Iowa State College through the courtesy of Dr. R. H. Porter. Seeds were germinated on moist filter paper in petri dishes for the study of the embryo and young primary root. Greenhouse grown plants were used for the study of the developing primary and young secondary roots. Older roots were obtained from field-grown plants. Roots were collected at daily intervals for the first seven days after planting, then at weekly intervals for twelve weeks.

Free-hand sections were made for diagnostic examination. The paraffin method was used for the study of histogenesis in the roots. The following killing solution was found to be satisfactory:

1 per cent acetic acid .....	75 c.c.
1 per cent chromic acid .....	20 c.c.
37 per cent formaldehyde .....	5 c.c.

<sup>1</sup>The writer wishes to express her sincere thanks for the advice and assistance given by Dr. J. E. Sass, under whose direction this work was conducted.

Materials were killed and hardened in this solution for at least 48 hours, dehydrated with an acetone-xylol series, and embedded in paraffin. The older and tougher materials were softened for sectioning by soaking mounted blocks in water at 35° C. for 12 to 24 hours. Sections were cut 10 or 12 microns thick and stained in hemalum and safranin, or safranin and fast green. Hemalum was found to be especially suitable for staining the sieve plates.

The drawings that illustrate the germination were made free-hand, and those that illustrate the vascular tissues were made either with a calibrated microprojector or by micrometer measurements.

## OBSERVATIONS

### A. Origin of Organs of the Seedling

The embryo of sorghum is a highly differentiated body. The plumule consists of a stem axis of five nodes and five leaf primordia, and the meristematic stem tip, all enclosed by the coleoptile. The scutellum is attached to one side of the axis. The broad region of attachment is known as the scutellar node. The first internode of the epicotyl is designated the "mesocotyl" by some authors. The hypocotyledonary portion of the axis consists of the radicle, encased in the conical coleorhiza.

Soon after being placed in soil or on moist paper at a temperature of 35° C., the grain absorbs water and begins to swell. In approximately 24 hours the coleorhiza bursts through the pericarp near the base of the caryopsis and tears a longitudinal slit in the pericarp (fig. 1). The emerging coleoptile has the form of a wedge (fig. 2).

After the coleorhiza has grown to a length of 2 millimeters, the primary root emerges, usually from one side of the coleorhiza (fig. 3). Unlike maize, oats, and wheat, sorghum has no adventitious roots at this stage. Root hairs arise from the epidermal cells just behind the region of elongation (fig. 4).

The coleoptile is pushed upward by the elongation of the first internode or "mesocotyl" (fig. 5 M). The coleoptile remains closed until its base is slightly below the surface of the soil, the tip of the coleoptile then splits on the side opposite the scutellum, and a foliage leaf emerges approximately a week after planting (fig. 6 L). The length of the mesocotyl varies with the depth of planting.

Approximately two or three days after planting, lateral roots begin to emerge from the primary root just above the root hair zone (figs. 5, 6 LR). In five-day-old seedlings the adventitious roots begin to develop, usually from the lower part of the mesocotyl (fig. 6 MR), though in a few cases some have been found on the upper part of the mesocotyl. After the adventitious roots of the coleoptile node and of the upper nodes become established, the primary root and the first adventitious roots become brown, about ten days after planting in the field and in three weeks in the greenhouse (figs. 7, 8 NR).

### B. Morphology of the Root System

The roots of sorghum may be divided into a temporary root system and a permanent root system. The temporary root system includes the primary root developed from the radicle and the adventitious roots developed on the mesocotyl. The primary root is the only seminal root in sorghum. Both the primary root and the adventitious roots of the mesocotyl produce lateral roots. The temporary roots are comparatively small and nearly uniform in diameter throughout their length. They comprise but a small part of the total root system.

The permanent root system includes the adventitious roots growing from the coleoptile node and from the several leaf nodes above the coleoptile node (figs. 7, 8, 9). The permanent adventitious roots have numerous branched lateral roots and are more fibrous than those of the maize plants. There are generally eight whorls of these nodal roots on a plant. The first whorl develops at the coleoptile node about ten days after planting in the field. The second, the third, and the fourth whorls arise successively at the succeeding upper nodes. In five-week-old field plants, when the inflorescence begins to differentiate, five whorls of the adventitious roots usually are present. When the inflorescence is nearly at the blooming stage six whorls usually are present. The seventh whorl develops at the time of pollination and the eighth whorl develops later.

The sixth, seventh, and eighth whorls arise above ground and may be designated brace roots (fig. 9). Chlorophyll often develops in the cortical regions of the aerial portions of brace roots. There are no lateral roots on the aerial portions, though many lateral roots develop from the underground portions in the same manner as on the roots of the underground whorls.

The size and the number of the roots at any node are approximately correlated with the size of the node from which the roots grow. The roots of the larger upper nodes are much larger and more numerous than those on the lower nodes.

### C. Histogenesis in the Root

#### 1. *Histology of the post-dormant radicle and of the primary root.*

The radicle of the sorghum embryo averages 1 millimeter in length, 800 microns in diameter near the scutellar plate, and tapers toward the growing point. The cell size and cell number increase gradually from the growing point toward the scutellar plate. The three histogens are clearly evident near the tip of the radicle (fig. 10). The periblem and dermatogen are developed from a single layer of meristematic cells. The cells of this layer divide periclinally; the outer daughter cells give rise to the dermatogen (fig. 10 D), whereas the inner derivatives produce the periblem (fig. 10 PE). The dermatogen becomes the epidermis; the periblem develops into the cortex. The plerome (fig. 10 PL), a central core of meristematic cells, develops into the tissues of the stele. The root cap

is produced by successive cell divisions of the root cap initial cells, immediately outside the periblem-dermatogen initial layer (fig. 10 RC).

Perceptible differentiation of elements of the vascular system can be recognized at approximately 60 microns behind the growing point of the emerging root. In a section at this level the plerome contains two large cells, having a diameter of about 20 microns, twice as large as the adjacent cells (fig. 11 LX). These cells enlarge rapidly and become vacuolate (figs. 12, 13 LX), and subsequently differentiate into "late-metaxylem" tubes.

The general arrangement of tissue systems is recognizable approximately 70 microns from the growing point, although these tissues have not attained structural maturity. A transverse section at this level shows the following details. The epidermis consists of radially elongated cells, averaging 18 microns in radial dimension and 6 to 8 microns tangentially. The cortex consists of 6 layers of compactly arranged cells, averaging 14 microns in diameter. The intercellular spaces between the cells are not pronounced. The endodermis consists of one layer of tangentially elongated cells. The pericyclic cells are approximately the same size as the adjacent cells of the stele. The early-metaxylem and protoxylem cells, which attain full differentiation before the precociously enlarged late metaxylem, are not distinguishable at this level. Phloem elements are not yet differentiated. There are at least two layers of root cap cells encircling the radicle at this level.

At 100 microns behind the growing point many stellar cells still exhibit meristematic activity. The cortex and stele are distinct. Near the periphery of the stele it is possible to identify the early-metaxylem cells, occurring as single cells, separated from each other and somewhat larger than the cells of the ground tissues (fig. 12 EX). The phloem regions consist of groups of two or three small cells alternating with the early-metaxylem initials (fig. 12 PH).

At a distance of 200 microns behind the apex these metaxylem cells are enlarged and form a radial pattern of nine points or arcs (rarely eight or ten) (fig. 20). At 276 microns behind the growing point, between each of the enlarged metaxylem points and the pericycle, one or two protoxylem cells appear, distinguishable by their more dense cytoplasm. If there are two such cells, they are always arranged radially (fig. 13 PX).

A transverse section 700 microns behind the growing point has the following features. The epidermis consists of one layer of cells, which average 35 microns in radial thickness and 12 microns in tangential length (fig. 14 EP). Encased by the epidermis is the cortex, which generally consists of six layers of cells at this level (fig. 14 C). The cells of the outer four layers are rounded, about 25 microns in diameter; those of inner two layers are radially flattened. Small intercellular spaces occur. The elliptical cells of the endodermis are about 22 microns in tangential length and 10 microns in radial length, with the tangential walls slightly convex (fig. 14 EN). The pericyclic cells are small, about one-

third as large as the endodermal cells (fig. 14 P). The two large late-metaxylem initial cells (fig. 14 LX) in the center are about 30 microns in diameter. Nine early-metaxylem initial cells (fig. 14 EX), rarely eight or ten, about 20 microns in diameter, are arranged in a circle inside the pericycle.

The differentiation of a xylem element consists of a progressive series of structural changes. The cell enlarges greatly in diameter, and the cytoplasm becomes vacuolate. When maximum enlargement has been attained, only a thin cytoplasmic layer lines the cell wall and a thin layer surrounds the nucleus, which is suspended by cytoplasmic strands (fig. 14 LX). Progressive thickening and lignification of the cell walls takes place. This typical sequence of differentiation progresses centripetally in the stele. The walls of the protoxylem and early-metaxylem cells in the oldest portion of the radicle become thickened almost simultaneously, approximately 36 hours after the seed is planted, and become lignified in a little over two days (fig. 15). The walls of the late-metaxylem at the above level do not become lignified in greenhouse plants until about two weeks after the seed is planted, whereas in the field lignification was found to take place in about one week. The undifferentiated stellar cells around the xylem elements may begin to develop into pitted tracheids in one-week-old greenhouse plants, but more commonly the walls become lignified in two weeks. The tracheids are short prismatic cells with oblique reticulate-pitted end walls and reticulate lateral pitting (figs. 16, 17 TR).

The development of the phloem is not as easy to work out as the development of xylem. Phloem cells are first recognized 100 microns behind the growing point. Between each pair of xylem arcs there are three small cells with dense cytoplasm. In cross section these cells are arranged in a triangle, with one cell toward the outside and two inside. The outer cell is smaller and rectangular, the inner two are larger and polygonal (fig. 12PH). By tracing from this level toward the growing point it is found that these three cells arise from one initial cell, which can be recognized 72 microns behind the growing point, but beyond this level the initial cell cannot be distinguished from the ground meristem. This phloem initial, which is slightly larger than the adjacent cells, first divides radially, more or less obliquely, into two cells, usually one larger than the other. The larger one divides again radially, somewhat obliquely. The triangular arrangement is the result of these divisions. At about 270 microns behind the growing point the outer small rectangular cell of each group begins to lose the cytoplasm and nucleus (fig. 13 PH). Finally, at 420 microns behind the growing point most of the small rectangular cells have become lysigenous cavities. During the development of the outer small rectangular cell each of the two inner cells divides into two daughter cells; one daughter cell becomes a sieve tube and the other forms a companion cell. It is not improbable that one or both of these two inner cells divide once more to form either more sieve tubes or more companion

cells, although details of subsequent division have not yet been determined. The sieve plates begin to develop two days after planting (fig. 15 SP). No phloem parenchyma can be identified in the primary root of sorghum.

Simultaneous differentiation of stelar tissues, and somewhat lagging differentiation of cortical tissues takes place, until characteristic structural maturity is attained. The cylindrical primary root is enclosed by the epidermis, a single layer of more or less uniform, vertically elongated, thin-walled cells. Epidermal hairs remain attached to the root long after the hairs have collapsed. Within the epidermis is the cortex consisting of 6-8 layers of wholly unspecialized parenchymatous cells with small intercellular spaces. In many grasses the outer layers of cortical cells become thick-walled, forming a firm hypodermis, but this does not occur in the primary root of sorghum. The innermost one or two layers of the cortex consist of regular, small, more or less square cells. In old plants the cortical tissues become brown, collapsed, and apparently dead, but the stele contains many cells with apparently living protoplasm during almost the whole life of the plant. The endodermis consists of a single layer of cells in which the upper, the lower, the radial, and the inner tangential walls are thickened and lignified. The tangential walls are pitted and possess protuberances which extend into the lumen of the cells. There are no passage cells. The pericycle contains two layers of radially elongated cells with uniformly thickened and pitted walls. The number of the protoxylem points is almost always nine, rarely eight or ten. Each protoxylem point consists of one or two vessels, which are comparatively small and have compact spiral secondary walls (figs. 15, 16, 17). Just within each protoxylem point is a single comparatively large metaxylem element. This element has thickened and uniformly pitted walls (figs. 15, 16, 17). Just within each protoxylem point is a single comparatively large metaxylem element. This element has thickened and uniformly pitted walls (fig. 15, 16, 17). The two large pitted metaxylem vessels in the center of the stele attain full differentiation much later than the other metaxylem elements (figs. 15, 16, 17 LX). Numerous reticulate tracheids occur between the xylem points and surrounding the phloem elements (figs. 16, 17 TR).

Alternating with the protoxylem arcs are nine phloem strands. Each phloem strand consists of 4-6 cells in transverse section, usually two sieve tubes and two companion cells. The sieve tubes are polygonal in cross section. The perforations of the sieve plates are comparatively large (figs. 15, 16). The companion cells usually are square in cross section. There is a lysigenous cavity on the outer side of each phloem group. The center of the stele is occupied by the two large late-metaxylem elements. There seems to be no true pith present.

The longitudinal aspect of the tissues of the radicle has received some attention in this study. In a longitudinal section most of the cells behind the growing point are rectangular in shape with the longer axis transverse.

The following description is based on the region 540 microns from the growing point. The epidermal cells are 30 microns in transverse dimension and 6 to 8 microns long. The cortex contains six layers of cells which are about 20 microns wide and 6 to 8 microns long. The two prominent zones inside the cortex are the endodermis and pericycle. The cells of these two tissues are much smaller and more compact than the cortical cells. Both the endodermal and pericyclic cells are about 10 microns wide and 6 to 8 microns in vertical length. The undifferentiated cells inside the pericycle are more or less square, about 12 microns across, and have dense cytoplasm. The early-metaxylem initial cells just inside the pericycle are about 20 microns in diameter and 12 microns long and have slightly thinner cytoplasm than those of the adjacent cells. The late-metaxylem initial cells, which form two strands in the center are larger, 30 microns in diameter, 20 microns long, and have thinner cytoplasm. The phloem region is distinct, but the identity of the elements is difficult to ascertain at this stage.

The above description applies to the corresponding terminal region of developing roots. In the older portions of the root, the secondary walls of the protoxylem vessels are compactly spiral, whereas those of the metaxylem vessels are pitted. The pits of the early-metaxylem walls are round and small, those of the late-metaxylem walls are more or less elliptical (fig. 17). The dissolution of the end walls of the vessels takes place during the lignification of the side walls.

## 2. *Origin and development of lateral roots and nodal roots.*

The lateral root originates in the pericycle directly outside of the phloem (fig. 18). Several pericyclic cells enlarge, develop dense cytoplasm, and then divide tangentially and radially, forming a conical growing point. The root primordium grows in diameter and elongates, the endodermis and cortex of the main root are ruptured, and finally the lateral root emerges from the older root.

Lateral roots develop histogens and primary tissues in the same manner as the radicle of the embryo. The mature structure of a lateral root is essentially like that of the main root from which the lateral root arises. The lateral root usually has 5 or 6 protoxylem points and one large metaxylem vessel in the center.

Adventitious nodal roots in general are similar to the lateral roots and primary roots with respect to histological differentiation and mature structure (fig. 21). The epidermis consists of vertically elongated cells. The thickness of the cortex increases with the size of the root. Usually there are nine layers of cells in the cortex of first whorl roots, and more layers in the upper whorls (fig. 21). During the thickening of protoxylem and early-metaxylem walls the walls of the outer few layers of the cortical cells also become thickened and pitted, forming a distinct hypodermal layer, whereas the cells of the inner layers remain thin-walled. There are two layers of thick-walled hypodermal cells in the first whorl roots.

and as many as five to seven layers in the roots of the fifth and upper whorls (figs. 19, 22, 23). The thin-walled cortical cells collapse in the older roots.

The endodermal cells become thickened and lignified in the fully differentiated adventitious roots as in the primary root, and exhibit abundant protuberances, which have been described as occurring in the endodermal cells of primary root. The pericycle is composed of two layers of cells with thickened and somewhat lignified cell walls (figs. 22, 23).

The number of the vascular arcs in each root increases with the size of the root. There are usually 20-30 protoxylem points and 8-12 late-metaxylem vessels in the first (lowest) whorl of roots. There are one or two protoxylem vessels and one, or rarely two, early-metaxylem vessels in one xylem arc. The spirally thickened walls of protoxylem and the uniformly pitted walls of the metaxylem are clearly shown in the longitudinal section. There are 4-6 cells in each of the phloem groups (fig. 23). Each phloem group usually contains one or two sieve tubes, two companion cells and a lysigenous cavity.

The following are the essential differences between the adventitious root and primary root:

1. The hypodermal cells of the adventitious root become thickened and lignified, whereas this differentiation does not occur in the primary root.
2. The endodermal and pericyclic cells of the adventitious roots have much thicker walls than those of the primary root.
3. There are more vascular arcs in the adventitious root than in the primary root.
4. The cell walls of phloem are thicker in the adventitious root than in the primary root.
5. The "pith" of the adventitious root consists of thin-walled parenchymatous cells, whereas in the primary root two large-metaxylem vessels occupy considerable space.

#### DISCUSSION

A comparison of the external morphology of the root systems of sorghum and other common cereals reveals some similarities and contrasts. The primary root of sorghum emerges from one side of the coleorhiza as in wheat. The elevation of the plumule by elongation of the mesocotyl, or first internode takes place as in maize, and differs from wheat, in which the elongation takes place in the second internode. The primary root is the only seminal root in sorghum, whereas there are two additional seminal roots in maize and oats, and four or five in wheat. Both sorghum and wheat produce some adventitious roots on the first internode. The permanent adventitious roots of sorghum are more fibrous and more profusely branched than those of maize.

Histogenesis and tissue organization in the roots of sorghum conform to the radial, endarch pattern of other Gramineae. The primary root of

sorghum has approximately nine xylem arcs, whereas maize has eighteen to thirty-six, and oats and wheat have eight or nine. Maize usually has five to seven large metaxylem tracheae and a central zone of parenchymatous pith; sorghum commonly has two large centrally located metaxylem tracheae.

The development of phloem has been worked out in more detail than in previous studies on the grasses. A single initial seems to give rise to each phloem strand by a unique series of divisions, whereby one derivative gives rise to a lysigenous cavity. A comparative study should be made to ascertain whether the histogenesis of phloem in the Gramineae follows a characteristic pattern, as does the development of xylem.

#### SUMMARY

1. A study was made of germination and root development of sorghum, *Holcus sorghum* var. *Kaoliang*.

2. The coleorhiza is the first structure to emerge from the caryopsis. When the coleorhiza is approximately 2 mm. long, the primary root bursts through the enveloping coleorhiza. The coleoptile then emerges and is pushed to the surface of the soil by elongation of the mesocotyl. Five or six days after germination adventitious roots appear on the lower part, and rarely on the upper part of the mesocotyl.

3. The primary or temporary root system consists of the primary root derived from the radicle and the adventitious roots on the mesocotyl. The secondary or permanent root system consists of the adventitious roots arising from the coleoptile node and several nodes above it. There are eight whorls of permanent nodal roots. The sixth, seventh and eighth whorls are designated brace roots. The size and the number of the adventitious roots of any node are correlated with the size of that node.

4. The origin of the histogens of the growing point of the radicle has been ascertained. The periblem and dermatogen are derived from a single layer of cells encasing the plerome initial cells. The cells of this encasing layer divide periclinally, producing two layers. The inner derivatives form the periblem, the outer layer constitutes the dermatogen. The plerome is derived from the plerome initial cells, which are easily distinguished from the periblem-dermatogen meristematic cells. The root cap initial cells at the outside of the periblem-dermatogen initial layer produce the root cap.

5. In the primary root the late-metaxylem initial cells can be recognized 60 microns behind the growing point, the early-metaxylem initial cells at 100 microns, and the protoxylem at 276 microns. The thickening and lignification of the walls of protoxylem and early-metaxylem take place simultaneously, whereas the walls of the late-metaxylem thicken and lignify much later. The protoxylem has compact spiral thickening; both the early-metaxylem and the late-metaxylem have pitted walls. Tracheids with reticulate thickenings are formed during the lignification of the late-metaxylem.

6. The phloem of the primary root begins to differentiate 72 microns

behind the growing point. Each phloem initial cell produces three cells arranged in a triangle. The outer cell gradually loses its protoplasm and becomes a lysigenous cavity, and the inner two cells divide again to form sieve tubes and companion cells.

7. During the differentiation of the vascular tissues the cell walls of the endodermis and pericycle become thickened and lignified.

8. There is no thick-walled hypodermis in the primary root, whereas such a zone is present in the permanent root.

9. Adventitious roots are similar to the primary root with respect to development and mature vascular structure, except that the adventitious roots have more xylem arcs.

10. The lateral roots arise from the pericycle of the main root and are structurally similar to the main root.

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#### EXPLANATION OF PLATES

##### PLATE I

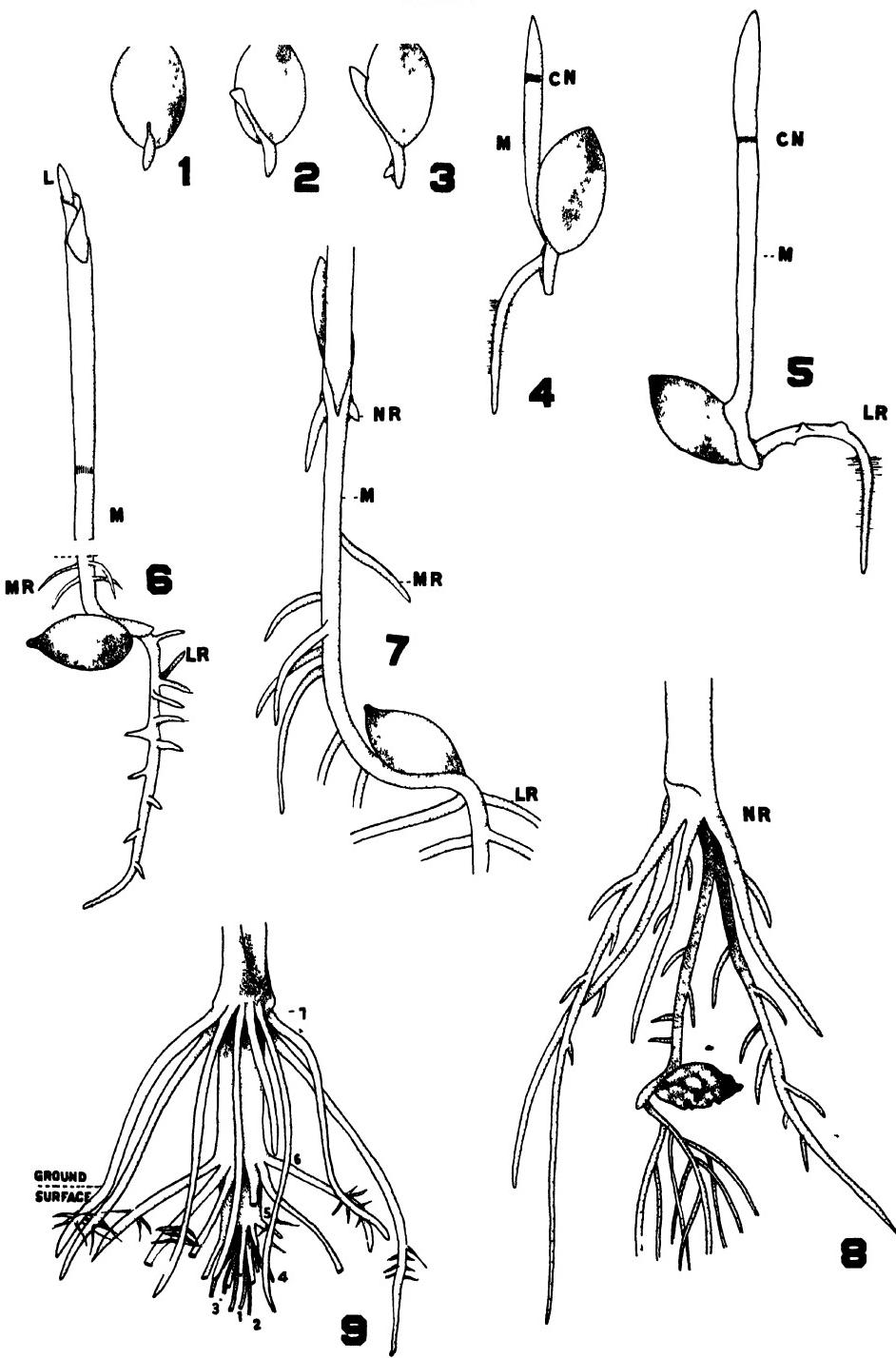
###### Figure:

1. 24-hour-old seedling, showing the coleorhiza emerging through the pericarp.  $\times 4$ .
2. 24-hour-old seedling, showing the emergence of the coleoptile.  $\times 4$ .
3. 36-hour-old seedling, showing the emergence of the primary root.  $\times 4$ .
4. 2-day-old seedling, showing the root hairs arising above the region of elongation.  $\times 4$ .
5. 3-day-old seedling, showing the elongation of the mesocotyl and the emergence of the lateral root.  $\times 4$ .
6. 6-day-old greenhouse-grown seedling, showing the split coleoptile, emerging leaves and adventitious root.  $\times 2\frac{1}{2}$ .
7. 3-week-old greenhouse-grown plant, showing the emergence of the adventitious roots from the coleoptile node.  $\times 2\frac{1}{2}$ .
8. 4-week-old greenhouse-grown plant, showing the temporary and permanent root system.  $\times 2$ .
9. 9-week-old field-grown plant, showing 7 whorls of adventitious roots.  $\times \frac{1}{2}$ .

C—cortex,  
CN—coleoptile node,  
D—dermatogen,  
EN—endodermis,  
EP—epidermis,  
EX—early metaxylem,  
L—foliage leaf,  
LR—lateral root,  
LX—late-metaxylem,  
M—mesocotyl,

MR—adventitious root on mesocotyl,  
NR—nodal roots (adventitious roots on nodes),  
P—pericycle,  
PE—periblem,  
PH—phloem,  
PL—plerome,  
PX—protoxylem,  
RC—root cap,  
SP—sieve plate,  
TR—tracheids.

## PLATE I

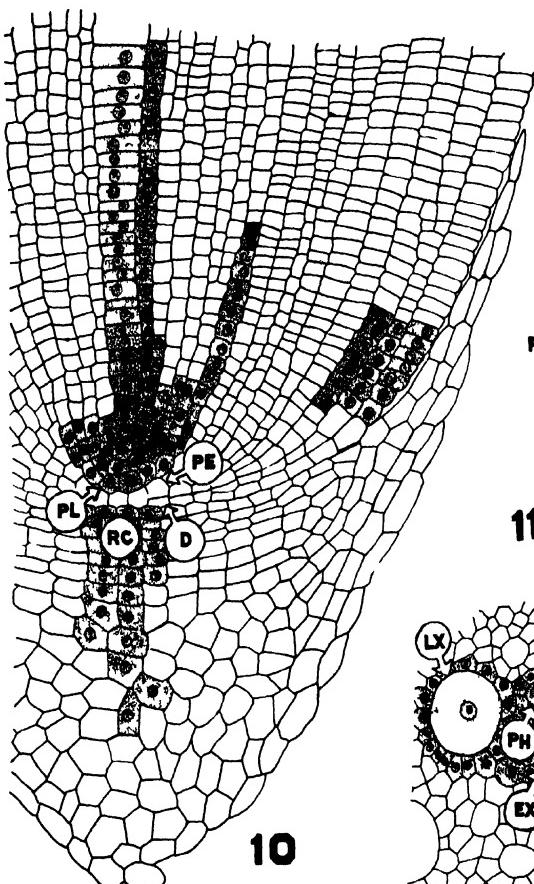


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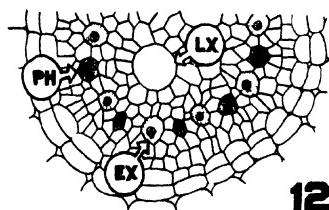
## PLATE II

10. Longitudinal section of the radicle tip of sorghum embryo.  $\times 270$ .
11. Cross section of a primary root, cut 60 microns behind the growing point, showing the initial cells of late-metaxylem.  $\times 270$ .
12. Cross section of a primary root, cut 100 microns behind the growing point, showing the early-metaxylem initial cells, and the phloem initial cells in successive stages of development.  $\times 270$ .
13. Cross section of a primary root, cut 270 microns behind the growing point, showing the initial cells of the protoxylem and phloem.  $\times 270$ .
14. Cross section of a primary root, cut 700 microns behind the growing point.  $\times 270$ .

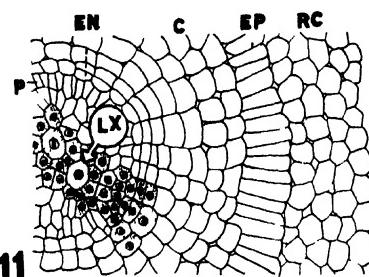
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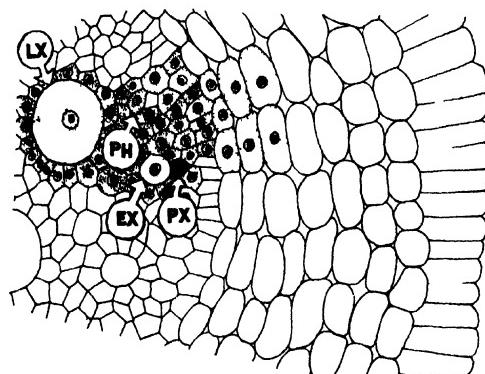
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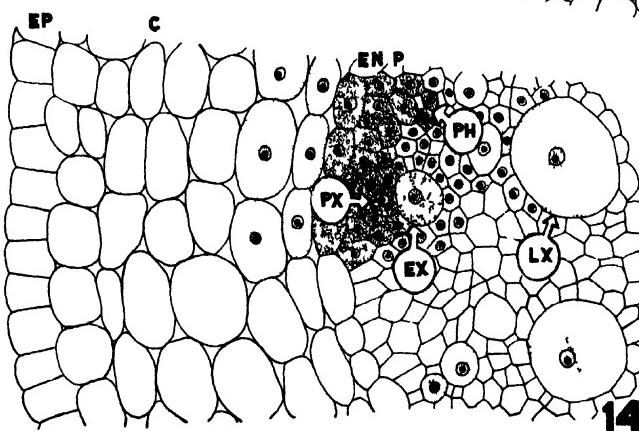
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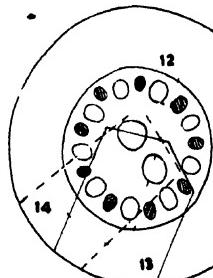
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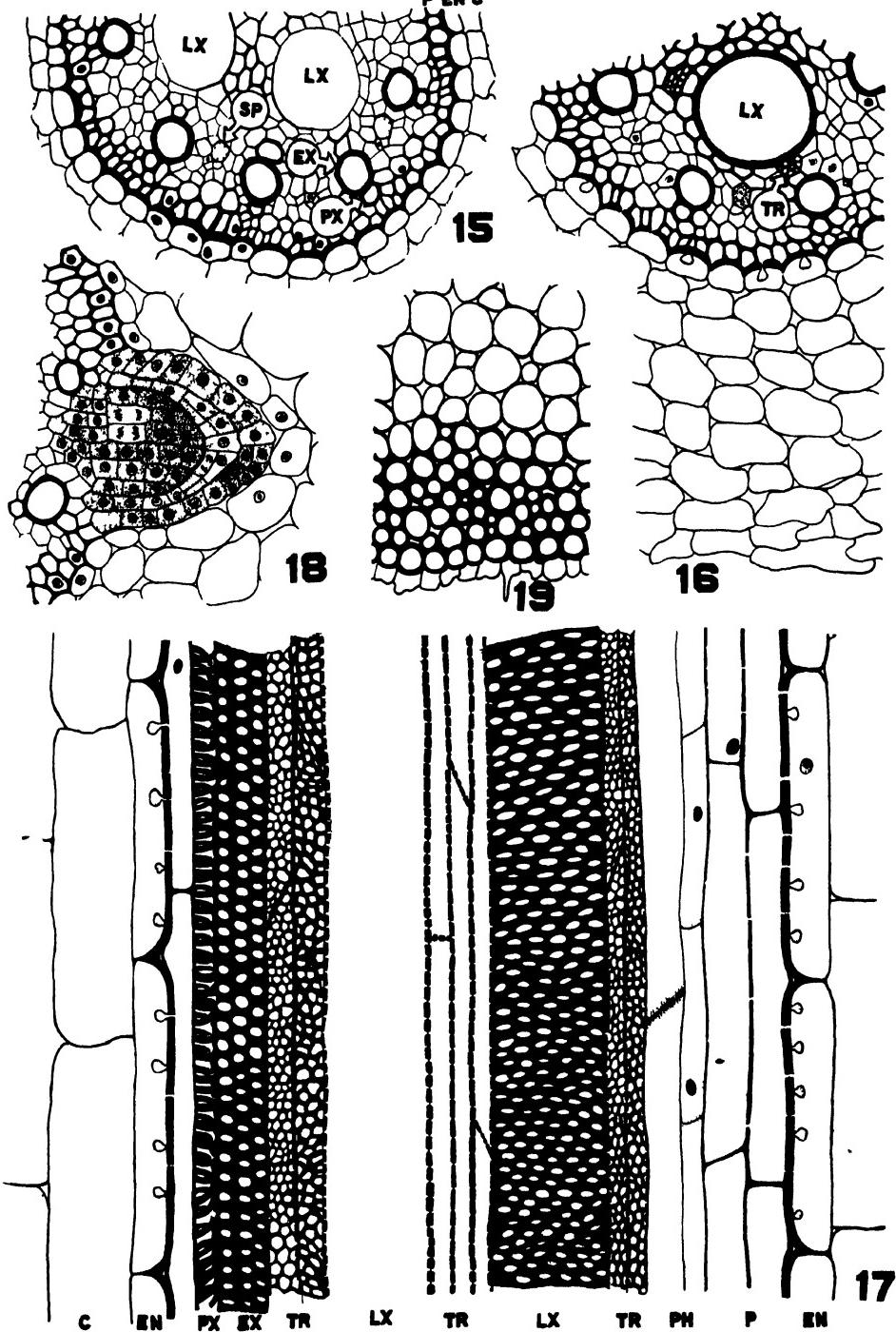


**PLATE III**

15. Cross section of a primary root of a 3-day-old plant.  $\times 270$ .
16. Cross section of a primary root of a 2-week-old plant, grown in greenhouse.  
 $\times 270$ .
17. Longitudinal section of a primary root of a 4-week-old greenhouse plant.  $\times 370$ .
18. Cross section of a primary root of a 7-day-old seedling, showing the lateral root arising from the pericycle, and the histogens of the lateral root tip.  $\times 270$ .
19. Cross section of the cortex and epidermis of an adventitious root of a field plant, showing the thick-walled hypodermis.  $\times 270$ .

PLATE III

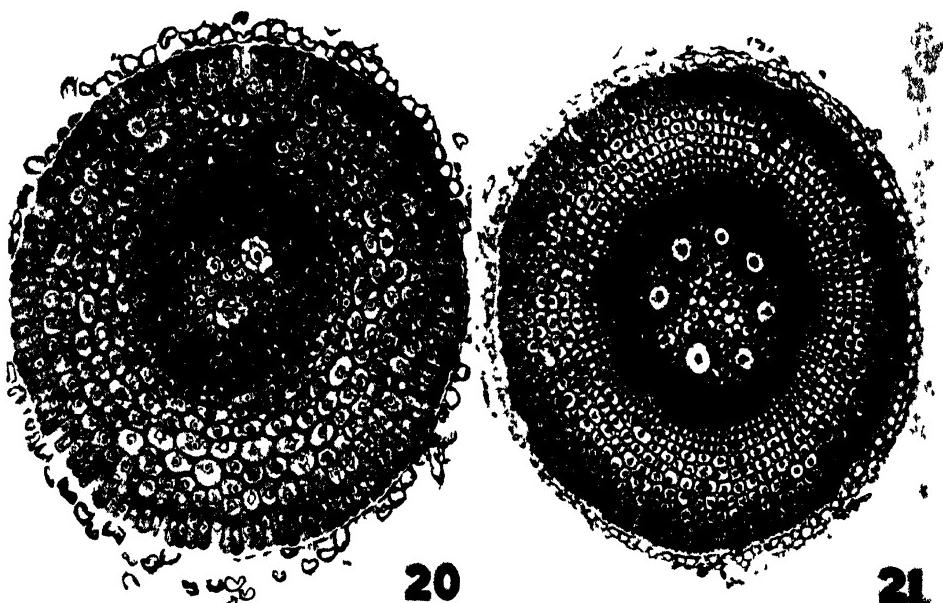
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## PLATE IV

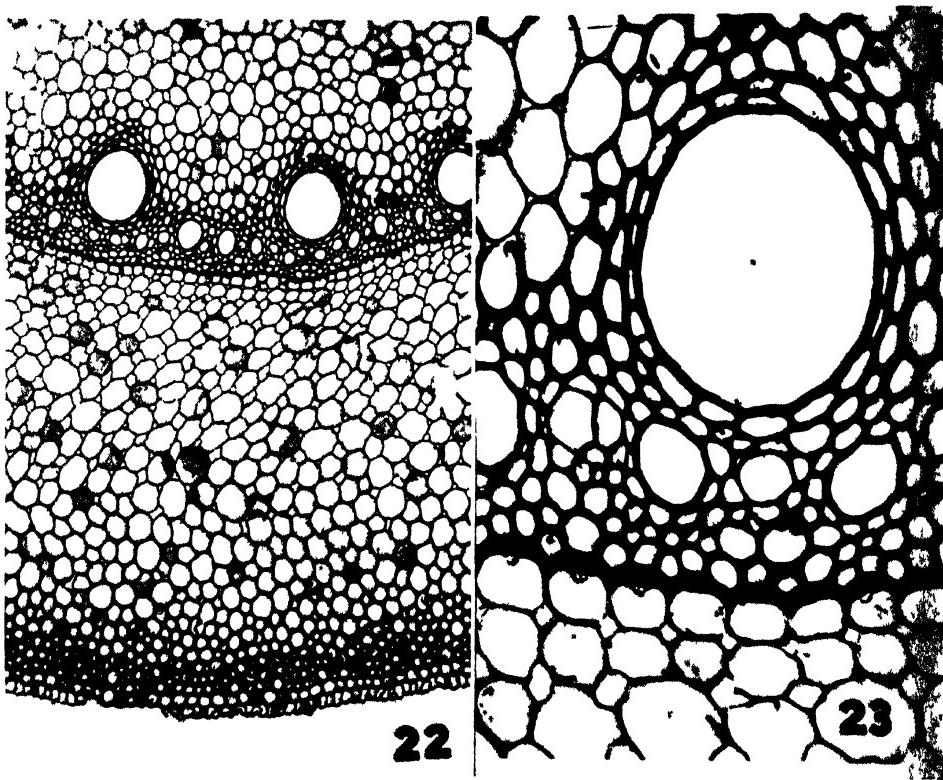
20. Tissue systems in a cross section of a primary root, cut 200 microns behind the growing point.  $\times 184$ .
21. Cross section of an adventitious root of the coleoptile node, cut 250 microns behind the growing point.  $\times 120$ .
22. Cross section of a brace root of a field plant.  $\times 120$ .
23. Detail from figure 22.  $\times 460$ .

PLATE IV



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22

23



# THE DETERMINATION OF THE TOTAL FIXED NITROGEN CONTENT OF POWER PLANT FLUE GASES

O. R. SWEENEY, F. E. CAMPBELL, AND BURDETTE JONES

*From the Chemical Engineering Department, Iowa State College*

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Although coal is known to contain approximately 1.4 per cent fixed nitrogen and about 604 million tons are burned every year, there is no evidence of any study as to what becomes of this fixed nitrogen. It is of course known that ammonia dissociates almost completely into hydrogen and nitrogen at 1,290° F., a temperature which is exceeded in power plant fires. This dissociation, however, occurs only if time is allowed for the reaction to reach equilibrium conditions which probably do not occur under the boilers. In the by-product coking of coal where this temperature is greatly exceeded, an average of 22 pounds of ammonium sulfate is obtained from a ton of coal. The presence under the boiler of large amounts of nitrogen from the air and the rapidity of the movement of the gases through the hot zones might be expected to prevent any considerable decomposition.

If all the fixed nitrogen in the coal burned annually could be recovered as ammonium sulfate it would amount to the staggering sum of 32 million tons valued at \$864,000,000. As sodium nitrate it would be 40.5 million tons valued at \$1,053,000,000.

If the nitrogen passed out of the stack as ammonia or nitrogen peroxide it would be washed out of the air by the rain and carried down to fertilize the soil. That nitrogen compounds produced in the air may be washed down into the soil has been shown by the work of Knight<sup>1</sup> at Cornell College, who found about 8 pounds of nitrogen a year per acre fixed by lightning discharge was washed down by rain and snow. If the fixed nitrogen from the coal were washed into the soil this would make the presence of large coal-using industries desirable in agricultural districts. Corrosion of iron and steel in the air is blamed by some authors on the nitrogen oxides in the air. Nothing seems to be actually known in regard to the truth of this assumption, so that it is desirable to secure data either confirming or disproving it.

To determine the amount of fixed nitrogen present in stack gases, analyses were made upon gases from one of the boilers at the Iowa State College power plant and from the boiler in the steam and gas laboratory of the Mechanical Engineering Department, Iowa State College. These results which were preliminary in nature and known to contain some inaccuracies showed very small amounts of fixed nitrogen present in the gases.

<sup>1</sup> Knight, N. Substances in rain and snow. Proc., Iowa Acad. Sci., 31: 325-26. 1918.

To secure more accurate data, samples of gas were collected and analyzed from Boiler No. 8, Lafayette Street Station of the East Division of the Iowa Public Service Company, Waterloo, Iowa. This boiler, which is used to supply steam at 200 pounds gauge pressure to turbines for the generation of electric power, was manufactured by the Combustion Engineering Company and was equipped with an underfeed stoker. It may be considered representative of the average power plant boiler.

Three sets of samples from this boiler were collected and analyzed.

TABLE 1  
SUMMARY OF RESULTS

Test No.	Location	Average Temperature °F.	Lb. Fixed Nitrogen per Lb. of Coal	Lb. Flue Gas per Lb. of Coal	Average Steam Flow Lb. per Hour
I	D	550	0.0000152	13.52	67,700
II	A	660	0.00001168	13.05	63,000
	B	585	0.00000468		
	C	520	0.00000404		
III	A	510	0.00000252	14.8	37,250
	B	450	0.00000246		
	C	395	0.00000267		

The first sample was secured from the last baffle plate, shortly ahead of the end of the boiler (D in Table 1) with the load on the boiler ranging from maximum to minimum. The second set of samples was secured at points A (second pass), B (end of boiler), and C (at stack), with the boiler operating at about two-thirds full load.

#### SAMPLING

The flue gas samples in tests II and III were removed from the boiler through standard sampling tubes, drawn through two Meyer glass sulfur bulbs, each having ten bulbs 1.25 inch in diameter containing conductivity water, in series with a 12-gallon aspirator bottle. The gas was sucked through the absorption bulb, by means of water flowing from the aspirator bottle, at approximately 250 cubic centimeters a minute. In run I, potash bulbs were used instead of sulfur bulbs. The volume of gas drawn out was determined by weighing the aspirator bottles at the end of the sampling period, filling with water, and weighing again. From the difference in weight (with necessary temperature correction) the volume was calculated.

At the end of the test, the water in the absorption bulbs was drained and the bulbs washed completely with conductivity water into glass stoppered bottles which were sealed with paraffin to prevent any loss of sample during transportation to the laboratory.

Several flue gas analyses were made during each run by the Orsat method and the average composition of the gases during the course of

TABLE 2  
COMPOSITION OF FLUE GAS

Constituents	TEST No.		
	I	II	III
Carbon dioxide, (CO <sub>2</sub> ) .....	14.0%	14.0%	12.6%
Oxygen, (O <sub>2</sub> ) .....	4.6%	1.6%	3.2%
Nitrogen, (N) .....	81.4%	83.9%	84.2%

the run determined. The hourly average of the steam was also taken. From the latter, the total water evaporated per day and the total coal burned per hour were calculated.

#### ANALYTICAL PROCEDURE

The samples together with washings of the bottles were transferred into clean 500 cc. distilling flasks and diluted to 200 cc. with conductivity water. Two strips of aluminum foil, 0.5 cm. by 10 cm., and 2 cc. of 20 per cent sodium hydroxide were added to each flask to produce hydrogen to reduce the nitrogen oxides to ammonia. The distilling flask was then connected to a glass condenser and distillation carried out at 6 to 10 cc. per minute. The condensate was collected in three 50 cc. Nessler tubes, treated with 1 cc. of standardized Nessler reagent, and compared with the standards to give total nitrogen content as ammonia.

All water used for final washing of apparatus and for dilution of the sample was ammonia, nitrate, and nitrite free water.

TABLE 3  
COMPOSITION OF COAL

	Percentage
Carbon, (C) .....	72.7
Oxygen, (O <sub>2</sub> ) .....	7.6
Hydrogen, (H <sub>2</sub> ) .....	5.2
Sulfur, (S) .....	3.6
Nitrogen, (N) .....	1.4
Ash .....	9.5
Total .....	100.0%

#### RESULTS

The data (Table 1) indicate that the fixed nitrogen content of the flue gases varied. This variation may have been caused by one or more of the following conditions which were not constant: the temperatures of the flue gases where sampled, the load on the boiler, the time required for the gases to pass through the system, and the main constituents of the flue gas, that is, carbon dioxide, oxygen, and nitrogen.

In test No. II the amount of fixed nitrogen decreased as the gas

passed through to the stack. This did not occur in test No. III. When test No. III was made the boiler was operating under a much lower load with consequent lower gas temperatures. The fact that nitrogen is fixed more readily at higher temperatures may account for the higher value secured in test No. II. In no case was the amount of fixed nitrogen in the gas more than 0.1085 per cent (the value for Test No. I) of the total available in the coal.

It should be worthwhile to study the possibility of increasing the amount of nitrogen fixed under boilers. The use of catalysts or changes in gas velocities at certain points might exert profound influence on the yield and be of tremendous economic importance to the nation.

# PRELIMINARY REPORT ON IOWA MOSQUITOES<sup>1</sup>

JOHN A. ROWE

From the Section of Entomology and Economic Zoology, Iowa Agricultural Experiment Station

Received April 15, 1941

This report on the mosquitoes of Iowa includes: (1) records of distribution and seasonal occurrence, (2) notes on larval habitats, and (3) larval associations. The data are based upon 605 collections distributed as follows: 1936, larval rearings 91, adult catches 2; 1939, larval rearings 77, adult catches 2; 1940, larval rearings 356, adult catches 77. The total monthly collections were: March 4, April 84, May 95, June 187, July 131, August 18, and September 86. Distributional records include both the county and nearest locality from which collections were taken. Monthly records are given for both larval and adult collections. Parenthesized numbers under "Larval records" indicate the percentage of the total monthly collections in which the species was taken. Where "Adult records" are given, these numbers mean the number of collections taken during the month. Only the major mosquito larval associations are given; numbers in parentheses refer to the number of times the association occurred.

Fifteen species of mosquitoes have been reported in scientific literature from Iowa. Dyar (3) published single locality records for *Aedes triseriatus* (Say), *Psorophora ciliata* (Fabr.), *Culex tarsalis* Coq., *Culex territans* Walk. (= *C. restuans* Theob.), *Culex testaceus* Van der Wulp (= *C. apicalis* Adams), *Culex salinarius* Coq., and *Mansonia perturbans* (Walk.). He also listed *Psorophora sayi* D. and K. (= *P. ferox* (Humb.)) from Algonquin (no date), H. J. Quayle; since there is no locality by this name in Iowa, this record may be from Illinois. In addition to the first three above mentioned species Hendrickson (5) reported *Theobaldia inornata* (Will.), *Culex pipiens* Linnaeus, *Aedes fitchii* (F. and Y.), *Aedes flavescens* (Müller), *Aedes nigromaculatus* (Ludlow), *Aedes sylvestris* (Theob.) (= *A. vexans* (Meigen)) *Aedes ? intrudens* Dyar. The last-named species was not taken during this investigation. Distributional notes on *Anopheles quadrimaculatus* Say, *Anopheles punctipennis* (Say) and *Anopheles walkeri* Theob. were reported by Bierring (1) based upon data taken during this survey.

Several Iowa species are known to be vectors of disease-producing organisms; *Anopheles quadrimaculatus* Say is an important vector of malaria, *Culex pipiens* Linnaeus transmits heartworm of dogs and bird malarias, *Aedes dorsalis* (Meig.) and *Aedes nigromaculatus* (Ludlow) are

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known to transmit equine encephalomyelitis experimentally, and *Aedes stimulans* (Walk.) is suspected in the spread of fowl-pox.

The writer is grateful to Dr. Walter L. Bierring and Dr. Carl Jordan of the Iowa State Department of Public Health for their active interest in mosquito survey work and for considerable financial support of these investigations. The work was done under the direction of Dr. C. J. Drake. Dr. R. M. Bailey, D. R. Lindsay, T. Y. Hsiao, Gaines Eddy and Lawrence Smith of Iowa State College have contributed specimens of several species.

In the use of names, the writer has followed Edwards (4). The publications of Bradley (2), and King and Bradley (6) were used for certain larval identifications.

#### Genus *Anopheles* Meigen

##### 1. *Anopheles maculipennis* Meigen

LOCALITIES: Allamakee Co.—Wexford; Butler Co.—New Hartford; Hamilton Co.—Jewell; Louisa Co.—Fruitland; Osceola Co.—Allendorf; Sac Co.—Lake View; Scott Co.—Le Claire.

LARVAL RECORDS: 7 collections; June (1.9), Aug. (12.5), Sept. (4.1).

LARVAL HABITATS: The collections were taken from ponded areas bordering streams in wooded and open country. The ponds were either permanent or intermittent and contained some marginal and aquatic floating plants.

ASSOCIATED LARVAE: *A. punctipennis* (7), *A. quadrimaculatus* (4), *C. tarsalis* (3), *C. apicalis* (2), *C. salinarius* (2), *U. sapphirina* (2).

##### 2. *Anopheles punctipennis* (Say)

LOCALITIES: Allamakee Co.—Harper's Ferry, Lansing, New Albin, Waukon Junction, Wexford; Audubon Co.—Audubon, Brayton, Hamlin; Benton Co.—Urbana; Blackhawk Co.—Waterloo; Boone Co.—Boone, Fraser; Bremer Co.—Frederika, Waverly; Buchanan Co.—Independence; Buena Vista Co.—Alta; Butler Co.—Clarksville, New Hartford, Shell-rock; Calhoun Co.—Pomeroy; Carroll Co.—Coon Rapids, Swan Lake State Park; Cass Co.—Atlantic, Lewis, Tranistan; Cedar Co.—Rochester; Cerro Gordo Co.—Mason City, Ventura; Cherokee Co.—Cherokee; Chickasaw Co.—Jericho, New Hampton; Clarke Co.—Osceola; Clay Co.—Dickens; Clayton Co.—Guttenburg, Marquette, St. Olaf; Clinton Co.—Folletts; Crawford Co.—Astor, Manilla; Davis Co.—Drakesville, Floris; Delaware Co.—Delhi, Manchester; Des Moines Co.—Burlington; Dickinson Co.—Milford; Dubuque Co.—Dubuque; Fayette Co.—Arlington, Elgin, Wadena; Floyd Co.—Charles City; Franklin Co.—Hampton; Fremont Co.—Sidney; Greene Co.—Grand Junction, Jefferson, Scranton; Guthrie Co.—Panora; Hamilton Co.—Jewell, Stanhope; Hancock Co.—Britt, Crystal Lake, Eagle Lake; Harrison Co.—Missouri Valley; Henry Co.—Mt. Pleasant; Howard Co.—Maple Leaf; Iowa Co.—Homestead, Ladora; Jackson Co.—Bellevue, Sabula; Johnson Co.—Lone Tree, Tiffin; Keokuk Co.—Hedrick, Sigourney; Lee Co.—Croton, Donnellson, Fort Madison,

Keokuk, Montrose, Sandusky, Wever; Louisa Co.—Fruitland, Grandview, Oakville, Wapello; Lucas Co.—Lucas; Mahaska Co.—Eddyville, Oskaloosa; Marshall Co.—Marshalltown; Monona Co.—Onawa; Monroe Co.—Albia, Hiteman; Muscatine Co.—Fairport, Nichols, Muscatine, Wilton Junction; Osceola Co.—Allendorf; Page Co.—Shenandoah; Palo Alto Co.—Ruthven; Polk Co.—Des Moines; Pottawattamie Co.—Council Bluffs, Oakland; Poweshiek Co.—Brooklyn; Sac Co.—Lake View; Scott Co.—Davenport, Le Claire, Montpelier; Sioux Co.—Alton; Story Co.—Ames; Tama Co.—Tama; Van Buren Co.—Cantril, Farmington; Wapello Co.—Ottumwa; Warren Co.—Indianola, Lake Ahquabi, Medford; Washington Co.—Ainsworth, Washington; Winnebago Co.—Forest City; Winneshiek Co.—Fort Atkinson; Woodbury Co.—Salix, Sioux City; Wright Co.—Dows, Galt.

**LARVAL RECORDS:** 178 collections; May (19.0), June (32.0), July (35.0), Aug. (50.0), Sept. (90.0).

**LARVAL HABITATS:** Almost all types of watered areas including temporary rain pools, pasture potholes (animal tracks), artificial receptacles, and shallow sheet flood water formed suitable breeding places. Some of the largest larval counts were from the grass-covered borders of small pasture streams, and from rocky or sandy pools in the beds of larger creeks. In these latter instances only *A. punctipennis* was usually found.

**ASSOCIATED LARVAE:** *C. tarsalis* (62), *C. apicalis* (53), *A. quadrimaculatus* (44), *C. salinarius* (40), *C. restuans* (31), *A. vexans* (29), *U. sapphirina* (24), *C. pipiens* (18), *C. erraticus* (18), *T. inornata* (17).

**ADULT RECORDS:** April (1), June (1), Aug. (2), Sept. (1). Unpublished records taken by Lawrence Smith from Clinton, Scott, Muscatine, and Louisa Counties include the following adult collections: July (5), Aug. (6), Sept. (6).

### 3. *Anopheles quadrimaculatus* Say

**LOCALITIES:** Audubon Co.—Hamlin; Blackhawk Co.—Waterloo; Boone Co.—Fraser; Butler Co.—Clarksville, New Hartford; Clarke Co.—Osceola; Clayton Co.—Guttenburg; Clinton Co.—Follettes; Davis Co.—Drakesville; Delaware Co.—Delhi, Manchester; Des Moines Co.—Burlington; Dubuque Co.—Dubuque; Guthrie Co.—Panora; Hamilton Co.—Jewell; Harrison Co.—Missouri Valley; Jackson Co.—Bellevue; Johnson Co.—Tiffin; Lee Co.—Fort Madison, Keokuk, Montrose; Louisa Co.—Fruitland, Grand View, Oakville, Wapello; Lucas Co.—Lucas; Mahaska Co.—Eddyville, Oskaloosa; Monroe Co.—Albia; Monona Co.—Onawa; Muscatine Co.—Muscatine, Nichols, Wilton Junction; Page Co.—Shenandoah; Pottawattamie Co.—Council Bluffs; Scott Co.—Davenport, Le Claire; Washington Co.—Crawfordsville; Woodbury Co.—Salix; Wright Co.—Cornelia, Galt.

**LARVAL RECORDS:** 61 collections; May (4.5), June (1.3), July (14.5), Aug. (50.0), Sept. (46.6).

**LARVAL HABITATS:** This important species was found chiefly in perma-

nent ponds which contained aquatic floating plants or debris. In a few instances in September, it was taken from weedy, evanescent rain ponds.

**ASSOCIATED LARVAE:** *A. punctipennis* (44), *C. tarsalis* (28), *C. apicalis* (24), *U. sapphirina* (19), *C. erraticus* (18), *C. salinarius* (13).

**ADULT RECORDS:** 2 collections, both in Aug. Records furnished by Lawrence Smith; July (6), Aug. (8), Sept. (7).

#### 4. *Anopheles walkeri* Theobald

**LOCALITIES:** Allamakee Co.—Lansing; Cerro Gordo Co.—Ventura; Dickinson Co.—Lake Park; Dubuque Co.—Dubuque; Hamilton Co.—Little Wall Lake; Louisa Co.—Fruitland; Monona Co.—Onawa; Muscatine Co.—Nichols; Poweshiek Co.—Brooklyn.

**LARVAL RECORDS:** 8 collections; May (1.1), June (1.3), July (0.8), Sept. (5.5).

**LARVAL HABITATS:** All larval collections were taken from permanent or intermittent marshes which supported dense growths of aquatic marginal plants such as *Typha* and *Sparganium*. The larvae in all instances showed the characters of the southern race of this species as figured by Bradley (1936).

**ASSOCIATED LARVAE:** *A. punctipennis* (6), *A. quadrimaculatus* (5), *C. apicalis* (3), *U. sapphirina* (3).

**ADULT RECORDS:** May (1), July (1), Aug. (1).

#### Genus *Uranotaenia* Lynch-Arribalzaga

##### 5. *Uranotaenia sapphirina* (Osten-Sacken)

**LOCALITIES:** Blackhawk Co.—Waterloo; Butler Co.—New Hartford; Cerro Gordo Co.—Ventura; Clay Co.—Dickens; Clinton Co.—Folletts; Davis Co.—Drakesville, Floris; Delaware Co.—Delhi, Manchester; Des Moines Co.—Augusta, Burlington; Dubuque Co.—Dubuque; Fayette Co.—Arlington; Hancock Co.—Britt; Henry Co.—Mt. Pleasant; Jackson Co.—Sabula; Lee Co.—Keokuk; Louisa Co.—Fruitland; Muscatine Co.—Muscatine, Wilton Junction; Polk Co.—Des Moines; Pottawattamie Co.—Council Bluffs; Scott Co.—Davenport, Le Claire.

**LARVAL RECORDS:** 32 collections; May (1.1), June (3.2), July (6.8), Aug. (37.5), Sept. (20.5).

**LARVAL HABITATS:** The larval habitats of this species resembled closely those of *A. quadrimaculatus*. Permanent ponds or permanent and intermittent marshes, containing considerable floating plants, produced the majority of the collections.

**ASSOCIATED LARVAE:** *A. punctipennis* (24), *A. quadrimaculatus* (19), *C. apicalis* (17), *C. tarsalis* (17), *C. salinarius* (9), *C. erraticus* (8).

#### Genus *Theobaldia* Neveu—Lemaire

##### 6. *Theobaldia inornata* (Williston)

**LOCALITIES:** Adair Co.—Greenfield; Adams Co.—Corning; Audubon Co.—Brayton, Exira, Hamlin; Benton Co.—Urbana; Blackhawk Co.—

Waterloo; Boone Co.—Boone, Fraser, Ledges State Park, Madrid; Bremer Co.—Frederika; Buena Vista Co.—Marathon, Storm Lake; Butler Co.—Clarksville; Carroll Co.—Dedham; Cass Co.—Atlantic, Griswold; Cerro Gordo Co.—Ventura; Clarke Co.—Osceola; Cherokee Co.—Larrabee; Clay Co.—Spencer; Clinton Co.—Buena Vista, De Witt, Follettes; Crawford Co.—Astor; Dallas Co.—Granger, Moran, Woodward; Decatur Co.—Leon; Des Moines Co.—Augusta; Dickinson Co.—Lake Park, Milford, Spirit Lake; Dubuque Co.—Dubuque; Emmet Co.—Wallingford; Fremont Co.—Farragut, Payne, Riverton; Greene Co.—Grand Junction, Jefferson; Guthrie Co.—Menlo, Panora; Hamilton Co.—Stratford; Hancock Co.—Britt, Crystal Lake; Harrison Co.—Missouri Valley; Humboldt Co.—Humboldt; Iowa Co.—Amana, Homestead; Jackson Co.—Preston; Jefferson Co.—Fairfield; Jones Co.—Anamosa, Oxford Mills, Wyoming; Kossuth Co.—St. Joseph; Lee Co.—Donnellson, Keokuk; Louisa Co.—Morning Sun, Wapello; Lyon Co.—Rock Rapids; Madison Co.—Winter-set; Marion Co.—Red Rock; Marshall Co.—Marshalltown; Mills Co.—Pacific Junction; Monona Co.—Onawa; Muscatine Co.—Conesville, Muscatine; Osceola Co.—Ocheyedan; Page Co.—Coin, Shenandoah; Palo Alto Co.—Emmetsburg, Ruthven; Plymouth Co.—Alton; Pocahontas Co.—Fonda; Polk Co.—Des Moines, Farrar; Pottawattamie Co.—(Carr's Lake) Council Bluffs, Oakland; Sac Co.—Lake View; Scott Co.—Dixon; Sioux Co.—Chatsworth; Story Co.—Ames, Collins, Colo; Tama Co.—Traer, Vining; Union Co.—Lorimer; Van Buren Co.—Keosauqua; Wapello Co.—Ottumwa; Warren Co.—Churchville, Norwalk; Webster Co.—Ft. Dodge; Winnebago Co.—Lake Mills, Rice Lake; Woodbury Co.—Sloan, Sioux City; Wright Co.—Cornelia.

**LARVAL RECORDS:** 121 collections; April (62.1), May (45.5), June (13.6), July (8.5), Sept. (11.0).

**LARVAL HABITATS:** Collections were taken from a large variety of habitats. During April and May larvae were found in spring pools, ponds, and stream bed pools resulting from melting snow or rain. In the summer and fall they were found in permanent ponds and in evanescent rain pools, which were often foul from stagnation or dumpage.

**ASSOCIATED LARVAE:** *A. vexans* (38), *C. tarsalis* (35), *C. salinarius* (20), *A. punctipennis* (17), *C. apicalis* (14), *C. restuans* (13). Pure cultures of *inornata* larvae were taken in 35 instances mostly during April.

**ADULT RECORDS:** April (1), May (4), June (1).

#### 7. *Theobaldia morsitans* (Theobald)

**LOCALITIES:** Osceola Co.—Ocheyedan; Clay Co.—Dickens.

**LARVAL RECORDS:** Only 2 larval collections were taken; both in June (1.3).

**LARVAL HABITATS:** 1 collection was taken from the permanent marshy border of Round Lake (Clay Co.); one from an intermittent marsh near Rush Lake (Osceola Co.).

**ASSOCIATED LARVAE:** *T. inornata* (2), *C. tarsalis* (2), *C. salinarius* (2), *C. restuans* (1), *A. vexans* (1).

Genus *Mansonia* Blanchard8. *Mansonia perturbans* (Walker)

Taken at only one locality: Muscatine, Muscatine Co., June 16 and 19 in a light-trap. Dyar (1922:31) reported this species from Ames.

Genus *Psorophora* R.—D.9. *Psorophora ciliata* (Fabricius)

**LOCALITIES:** Benton Co.—Belle Plaine; Calhoun Co.—Pomeroy; Cass Co.—Atlantic; Delaware Co.—Manchester; Des Moines Co.—Burlington; Dubuque Co.—Dubuque; Greene Co.—Scranton; Guthrie Co.—Bagley, Guthrie Center, Panora; Hardin Co.—Hubbard; Iowa Co.—Homestead; Jackson Co.—Sabula; Johnson Co.—Lone Tree; Monona Co.—Onawa; Page Co.—Clarinda, Hepburn, Shenandoah; Plymouth Co.—Merrill, Remsen; Pocahontas Co.—Fonda; Sac Co.—Early, Lake View; Story Co.—Ames; Woodbury Co.—Sergeant Bluff, Sioux City.

**LARVAL RECORDS:** 21 collections; May (2.2), June (0.3), July (10.2), Sept. (1.3).

**LARVAL HABITATS:** About 80 per cent of the larval collections were taken from temporary rain pools, stream bed pools, pasture potholes (animal tracks), and flooded areas. About 20 per cent were from margins of marshes and ponds.

**ASSOCIATED LARVAE:** *A. vexans* (15), *A. punctipennis* (6), *C. tarsalis* (6), *A. trivittatus* (6), *C. restuans* (4).

**ADULT RECORDS:** June (4), July (1), Aug. (4), Sept. (1); in a few instances, females in considerable numbers were biting fiercely.

10. *Psorophora horrida* (D. and K.)

**LOCALITIES:** Clinton Co.—Folletts; Dubuque Co.—Dubuque; Page Co.—Shenandoah; Story Co.—Ames; Washington Co.—Washington; Woodbury Co.—Sioux City.

**LARVAL RECORDS:** 2 collections; taken only in July (0.8) and Sept. (1.3).

**LARVAL HABITATS:** Both larval collections were from temporary rain pools in wooded areas.

**ASSOCIATED LARVAE:** *A. vexans* (2), *A. trivittatus* (1), *A. punctipennis* (1), *C. salinarius* (1).

**ADULT RECORDS:** June (2), July (1), Aug. (3), Sept. (1); females were usually encountered in or near woods; they bite viciously.

11. *Psorophora ferox* (Humboldt)

**LOCALITIES:** Dubuque Co.—Dubuque; Page Co.—Shenandoah; Story Co.—Ames; Wapello Co.—Ottumwa.

**LARVAL RECORDS:** A single larval collection was taken in June (1.1).

**LARVAL HABITAT.** Taken from a temporary pothole in a creek bed in thick woods.

**ASSOCIATED LARVAE:** *A. punctipennis*, *A. trivittatus*, *A. canadensis*, *A. vexans*, *C. restuans*.

**ADULT RECORDS:** June (1), July (2); females were quite abundant and annoying in wooded areas south of Dubuque.

**12. *Psorophora columbiae* (D. and K.)**

One larval collection was taken: Ogden, Boone Co., July 8; from a temporary rain pool in an open meadow.

**ASSOCIATED LARVAE:** *A. vexans*, *C. tarsalis*, *C. restuans*.

**13. *Psorophora signipennis* (Coquillett)**

**LOCALITIES:** Cass Co.—Atlantic; Guthrie Co.—Bagley; Harrison Co.—Missouri Valley; Monona Co.—Onawa, Whiting; Page Co.—Shenandoah; Plymouth Co.—Akron, Merrill, Westfield; Pottawattamie Co.—Oakland; Sioux Co.—Chatsworth; Woodbury Co.—Sergeant Bluff, Sioux City.

**LARVAL RECORDS:** 7 collections; June (0.6), July (5.1).

**LARVAL HABITATS:** 6 collections were taken from temporary rain pools and flooded areas; one was from an intermittent marsh.

**ASSOCIATED LARVAE:** *A. vexans* (5), *A. trivittatus* (3), *P. ciliata* (2), *C. tarsalis* (2).

**ADULT RECORDS:** June (6), July (1), Aug. (2); females are vicious biters, and were encountered in considerable numbers on a few occasions.

**Genus *Aedes* Meigen**

**14. *Aedes nigromaculis* (Ludlow)**

**LOCALITIES:** Appanoose Co.—Centerville; Audubon Co.—Hamlin; Cass Co.—Atlantic; Harrison Co.—Missouri Valley (Hendrickson 1930: 127); Monona Co.—Onawa, Whiting; Osceola Co.—Sibley; Page Co.—Shenandoah; Plymouth Co.—Akron, Remsen, Westfield; Sioux Co.—Alton; Story Co.—Ames; Woodbury Co.—Salix, Sergeant Bluff, Sioux City.

**LARVAL RECORDS:** 9 collections: May (2.2), June (1.9), July (2.5), Sept. (1.4).

**LARVAL HABITATS:** 6 collections were from temporary rain pools, pasture potholes (animal tracks), and flooded areas; 3 were from intermittent stream bed pools and marshes.

**ASSOCIATED LARVAE:** *A. vexans* (7), *A. trivittatus* (3), *C. tarsalis* (3), *T. inornata* (2).

**ADULT RECORDS:** June (4), July (1).

**15. *Aedes fitchii* (F. and Y.)**

**LOCALITIES:** Blackhawk Co.—Waterloo; Clay Co.—Spencer; Jackson Co.—Bellevue, Hurstville; O'Brien Co.—Primghar; Winnebago Co.—Buffalo Center (Hendrickson 1930: 127).

**LARVAL RECORDS:** 5 collections: April (4.8), June (0.6).

**LARVAL HABITATS:** 4 collections were from temporary spring pools; one was from an intermittent marshy area.

**ASSOCIATED LARVAE:** *T. inornata* (2), *A. vexans* (1), *A. stimulans* (1).

**16. *Aedes flavescens* (Müller)**

This species was taken, as adults, from one locality: Lake Mills, Winnebago Co., May 31. Hendrickson (1930:127) reported an adult from the same county 7 mi. NW Thompson.

**17. *Aedes riparius* D. and K.**

**LOCALITIES:** Clinton Co.—Elwood; Grundy Co.—Grundy Center; Hamilton Co.—Jewell.

**LARVAL RECORDS:** 3 collections: April (2.4), June (0.6).

**LARVAL HABITATS:** One collection was from a temporary spring pool, one from temporary pasture potholes and one from spring run-off water in an intermittent marsh.

**ASSOCIATED LARVAE:** *A. vexans* (2), *A. impiger* (1).

**18. *Aedes stimulans* (Walker)**

**LOCALITIES:** Boone Co.—Fraser; Cedar Co.—Rochester; Clayton Co.—Marquette; Clinton Co.—Folletts; Fremont Co.—Percival; Jackson Co.—Bellevue; Jones Co.—Oxford Mills; Linn Co.—Waubeek; Scott Co.—McCausland.

**LARVAL RECORDS:** 6 collections; March (100), April (4.8), May (1.1).

**LARVAL HABITATS:** 5 collections were from temporary waters of spring pools and pond overflows; one was from an intermittent stream bed pool.

**ASSOCIATED LARVAE:** *A. impiger* (2), *A. fitchi* (1), *A. canadensis* (1), *A. sticticus* (1).

**ADULT RECORDS:** June (3), Aug. (1).

**19. *Aedes campestris* D. and K.**

**LOCALITIES:** Plymouth Co.—Akron; Osceola Co.—Sibley; Monona Co.—Whiting.

**LARVAL RECORDS:** 1 larval collection was taken from temporary flood water in June. *A. vexans* was present in the same water.

**ADULT RECORDS:** 2 collections in June.

**20. *Aedes canadensis* (Theobald)**

**LOCALITIES:** Boone Co.—Fraser; Des Moines Co.—Augusta; Dubuque Co.—Dubuque; Hamilton Co.—Strafford; Henry Co.—Lockridge; Jones Co.—Wyoming; Monroe Co.—Albia; Wapello Co.—Ottumwa.

**LARVAL RECORDS:** 9 collections; March (100), April (3.6), May (5.6).

**LARVAL HABITATS:** 8 collections were taken from temporary or intermittent waters of spring pools and stream bed pools; one was from artificial receptacles floating in a city dumping pond.

**ASSOCIATED LARVAE:** *T. inornata* (8), *A. punctipennis* (3), *C. restuans* (3), *C. salinarius* (3), *A. vexans* (2).

**21. *Aedes dorsalis* (Meigen)**

**LOCALITIES:** Buena Vista Co.—Marathon, Storm Lake; Clarke Co.—Osceola; Dickinson Co.—Lake Park; Dubuque Co.—Dubuque; Monona Co.—Onawa, Whiting; Palo Alto Co.—Ruthven.

**LARVAL RECORDS:** 7 collections; April (1.2), May (1.1), June (1.9), July (1.7).

**LARVAL HABITATS:** 5 collections were taken from temporary waters of stream bed pools, pasture potholes, and flooded areas; 2 were from fluctuating marginal areas of marshes.

**ASSOCIATED LARVAE:** *A. vexans* (7), *T. inornata* (3), *A. punctipennis* (2), *C. tarsalis* (2), *C. restuans* (2).

**ADULT RECORDS:** June (4).

## 22. *Aedes trivittatus* (Coquillett)

**LOCALITIES:** Audubon Co.—Audubon, Brayton, Hamlin; Buena Vista Co.—Storm Lake; Cass Co.—Atlantic; Clayton Co.—Marquette; Clinton Co.—Follets; Crawford Co.—Charter Oak; Delaware Co.—Manchester; Dubuque Co.—Dubuque; Franklin Co.—Hampton; Greene Co.—Grand Junction; Hamilton Co.—Ellsworth; Harrison Co.—Mondamon; Jackson Co.—Baldwin; Jones Co.—Oxford Mills; Mahaska Co.—New Sharon; Monona Co.—Onawa; Page Co.—Shenandoah; Plymouth Co.—Merrill, Westfield; Pottawattamie Co.—Oakland; Sac Co.—Early, Lake View; Scott Co.—Davenport, McCausland; Sioux Co.—Alton, Oak Grove State Park; Story Co.—Ames; Woodbury Co.—Sioux City.

**LARVAL RECORDS:** 17 collections; May (2.2), June (3.2), July (5.9), Sept. (4.1).

**LARVAL HABITATS:** 13 collections were from temporary flooded areas, rain pools and stream bed pools; 2 from intermittent marshes, and 2 from the margins of permanent ponds.

**ASSOCIATED LARVAE:** *A. vexans* (12), *A. punctipennis* (6), *P. ciliata* (6), *C. tarsalis* (6), *T. inornata* (3), *P. signipennis* (3), *A. nigromaculis* (3), *C. restuans* (3).

**ADULT RECORDS:** June (9), July (5), Aug. (6), Sept. (7). This species is an important pest during the summer and fall.

## 23. *Aedes aurifer* (Coquillett)

One adult collection was taken in a wooded area near Marquette, Clayton Co., June 26.

## 24. *Aedes impiger* (Walker)

**LOCALITIES:** Boone Co.—Fraser; Buena Vista Co.—Marathon; Linn Co.—Waubeek; Pocahontas Co.—Fonda.

**LARVAL RECORDS:** 4 collections; March (100), April (3.6).

**LARVAL HABITATS:** 3 collections were from temporary spring pools; one was from the margins of an intermittent marsh.

**ASSOCIATED LARVAE:** *A. vexans* (3), *A. stimulans* (2), *A. canadensis* (1), *T. inornata* (1).

## 25. *Aedes spenceri* (Theobald)

This species was taken from a single locality: Panora, Guthrie Co., April 15. Larvae were found in a temporary spring pool in open country. *T. inornata* was taken in the same pool.

**26. *Aedes sticticus* (Meigen)**

This species is also known as *A. hirsuteron* (Theob.)

LOCALITIES: Allamakee Co.—Waukon Junction; Clayton Co.—Marquette; Clinton Co.—Camanche, Folletts; Dubuque Co.—Dubuque; Hamilton Co.—Stratford; Jackson Co.—Bellevue; Jones Co.—Oxford Mills; Muscatine Co.—Muscatine.

LARVAL RECORDS: One larval collection; taken from a temporary spring pool in thick woods, April 27. *T. inornata*, *A. stimulans* and *A. canadensis* were taken in the same pool.

ADULT RECORDS: May (1), June (6), July (1).

**27. *Aedes triseriatus* (Say)**

LOCALITIES: Clayton Co.—Bellevue; Clinton Co.—Folletts; Floyd Co.—Charles City; Franklin Co.—Hampton; Hancock Co.—Crystal Lake; Iowa Co.—Amana (Hendrickson 1930: 127); Page Co.—Shenandoah; Story Co.—Ames; Woodbury Co.—Sioux City.

ADULT COLLECTIONS: The records above are biting-adult collections; taken May (1), July (1), Aug. (1), Sept. (5). This species is known to breed in tree holes and artificial containers.

**28. *Aedes vexans* (Meigen)**

LOCALITIES: Adams Co.—Corning; Allamakee Co.—Lansing, New Albin, Waukon Junction; Appanoose Co.—Centerville; Audubon Co.—Brayton, Hamlin; Benton Co.—Belle Plaine; Blackhawk Co.—Waterloo; Boone Co.—Boone, Ogden; Buchanan Co.—Winthrop; Buena Vista Co.—Alta, Marathon, Storm Lake; Butler Co.—Allison, Clarksville, Shell Rock; Carroll Co.—Carroll, Swan Lake State Park; Cass Co.—Atlantic; Cedar Co.—Durant; Cherokee Co.—Cherokee, Larrabee; Clarke Co.—Osceola; Clay Co.—Dickens, Spencer, Trumble Lake; Clayton Co.—Marquette, St. Olaf; Clinton Co.—Calamus, Clinton, De Witt, Elwood, Folletts, Goose Lake, Haowntown; Dallas Co.—Granger, Moran, Woodward; Delaware Co.—Manchester, Masonville; Dickinson Co.—Superior; Dubuque Co.—Dubuque, Worthington; Floyd Co.—Charles City; Franklin Co.—Hampton; Fremont Co.—Payne; Greene Co.—Grand Junction, Scranton; Guthrie Co.—Guthrie Center, Jamaica, Panora; Hamilton Co.—Ellsworth, Jewell, Stanhope, Stratford; Hancock Co.—Britt, Crystal Lake; Hardin Co.—Ackley; Harrison Co.—Missouri Valley, Mondamon; Humboldt Co.—Humboldt; Iowa Co.—Homestead; Jackson Co.—Bellevue, Monmouth, Preston, Sabula; Jefferson Co.—Fairfield; Johnson Co.—Lone Tree; Jones Co.—Anamosa, Oxford Mills; Kossuth Co.—St. Joseph; Lee Co.—Keokuk; Linn Co.—Cedar Rapids, Central City; Louisa Co.—Columbus Junction, Wapello; Madison Co.—Winterset; Mahaska Co.—New Sharon; Marion Co.—Red Rock; Marshall Co.—Marshalltown; Monona Co.—Onawa, Whiting; Monroe Co.—Lovilia; Muscatine Co.—Muscatine; O'Brien Co.—Primghar; Osceola Co.—Sibley; Page Co.—Clarinda, Shenandoah; Palo Alto Co.—Ayrshire, Emmetsburg, Mallard, Ruthven, Silver Lake; Plymouth Co.—Akron, Le Mars, Merrill, Remsen, Westfield; Poca-

hontas Co.—Fonda, Pocahontas; Polk Co.—Farrar; Pottawattamie Co.—Council Bluffs, Oakland; Sac Co.—Early, Lake View; Scott Co.—Davenport, Dixon, McCausland, Princeton; Sioux Co.—Alton, Chatsworth; Story Co.—Ames, Colo; Tama Co.—Vining; Union Co.—Creston, Lormor; Wapello Co.—Ottumwa; Warren Co.—Churchville; Washington Co.—Washington; Wayne Co.—Corydon; Webster Co.—Ft. Dodge; Winnebshiek Co.—Decorah, Ft. Atkinson; Woodbury Co.—Brown's Lake, Salix, Sioux City, Sloan.

LARVAL RECORDS: 154 collections; April (47.5), May (26.1), June (30.0), July (31.4), Aug. (12.5), Sept. (9.6).

LARVAL HABITATS: Larvae were taken generally in temporary waters resulting from rains or floods. In many instances during June, August, and September, large broods of larvae were produced. Considerable breeding was also found around the margins of permanent ponds, lakes, and marshes due to the fluctuation of the water levels caused by rains.

ASSOCIATED LARVAE: *T. inornata* (38), *C. tarsalis* (31), *A. punctipennis* (29), *C. restuans* (20), *C. salinarius* (16), *P. ciliata* (15), *A. trivittatus* (12). Apparently pure populations of *vexans* were taken in 51 instances.

ADULT RECORDS: May (2), June (20), July (10), Aug. (8), Sept. (4). This species is without doubt the major pest mosquito of the state.

## 29. *Aedes cinereus* Meigen

LOCALITIES: Buchanan Co.—Winthrop; Clarke Co.—Osceola; Jackson Co.—Preston; Jefferson Co.—Fairfield; Louisa Co.—Columbus Junction; Palo Alto Co.—Emmetsburg.

LARVAL RECORDS: 6 collections; April (6.1), July (0.8).

LARVAL HABITATS: 4 collections were from temporary spring pools; one from the margin of a permanent pond; and one from a permanent marsh.

ASSOCIATED LARVAE: *A. vexans* (5), *T. inornata* (2), *A. dorsalis* (1), *C. tarsalis* (1).

## Genus *Culex* Linnaeus

### 30. *Culex apicalis* Adams

LOCALITIES: Allamakee Co.—New Albin, Wexford; Benton Co.—Urbana; Blackhawk Co.—Waterloo; Boone Co.—Boone, Fraser; Bremer Co.—Frederika, Shell Rock, Waverly; Buchanan Co.—Independence; Buena Vista Co.—Storm Lake; Butler Co.—Allison; Cass Co.—Tranistan; Cerro Gordo Co.—Ventura; Clarke Co.—Osceola; Clayton Co.—Guttenburg, Marquette; Clinton Co.—Folletts; Davis Co.—Drakesville; Delaware Co.—Delhi; Des Moines Co.—Burlington; Dickinson Co.—Milford; Dubuque Co.—Dubuque, Worthington; Emmet Co.—Wallingford; Fayette Co.—Arlington; Floyd Co.—Charles City; Franklin Co.—Hampton; Fremont Co.—Sidney; Greene Co.—Grand Junction; Hamilton Co.—Jewell; Harrison Co.—Missouri Valley; Henry Co.—Mt. Pleasant; Jackson Co.—Sabula; Jones Co.—Oxford Mills; Lee Co.—Croton, Donnellson,

Fort Madison, Keokuk, Montrose, Sandusky, Wever; Louisa Co.—Fruitland, Grand View, Oakville; Monona Co.—Onawa; Monroe Co.—Albia; Muscatine Co.—Muscatine, Nichols; Page Co.—Coin; Pottawattamie Co.—Council Bluffs; Sac Co.—Lake View; Scott Co.—Davenport, McCausland; Story Co.—Ames; Van Buren Co.—Farmington; Wapello Co.—Ottumwa; Washington Co.—Crawfordsville; Woodbury Co.—Brown's Lake, Sioux City; Wright Co.—Cornelia, Galt.

**LARVAL RECORDS:** 84 collections; April (1.2), May (12.4), June (22.1), July (9.3), Sept. (37.0).

**LARVAL HABITATS:** Permanent ponds and marshes and intermittent marshes produced most of the larval collections. In the fall it was found on a few occasions in evanescent rain pools.

**ASSOCIATED LARVAE:** *A. punctipennis* (53), *C. tarsalis* (35), *A. quadrimaculatus* (24), *C. salinarius* (22), *U. sapphirina* (17).

### 31. *Culex tarsalis* Coquillett

**LOCALITIES:** Allamakee Co.—Lansing; Audubon Co.—Audubon, Brayton, Hamlin; Benton Co.—Urbana; Blackhawk Co.—Waterloo; Boone Co.—Boone, Fraser, Ogden; Bremer Co.—Frederika, Waverly; Buena Vista Co.—Marathon; Butler Co.—Clarksville, New Hartford, Shell Rock; Carroll Co.—Carroll, Dedham, Manning, Swan Lake State Park; Cass Co.—Atlantic, Griswold, Lewis, Lorah, Tranistan; Cerro Gordo Co.—Ventura; Cherokee Co.—Cherokee; Chickasaw Co.—Jericho, New Hampton; Clarke Co.—Osceola; Clay Co.—Dickens; Clayton Co.—Guttenburg, Marquette, Sabula; Clinton Co.—Folletts; Crawford Co.—Astor, Manilla; Davis Co.—Floris; Decatur Co.—Leon; Delaware Co.—Augusta, Burlington; Dickinson Co.—Lake Park; Dubuque Co.—Dubuque; Floyd Co.—Charles City; Fremont Co.—Sidney; Greene Co.—Grand Junction, Jefferson, Scranton; Guthrie Co.—Panora; Hamilton Co.—Ellsworth; Hancock Co.—Britt, Crystal Lake; Harrison Co.—Little Sioux, Missouri Valley; Henry Co.—Mt. Pleasant; Iowa Co.—Homestead; Keokuk Co.—Richland, Sigourney; Lee Co.—Donnellson, Fort Madison, Keokuk, Montrose; Linn Co.—Central City; Louisa Co.—Fruitland, Oakville, Wapello; Lyon Co.—Lester, Rock Rapids; Madison Co.—Winterset; Mahaska Co.—Eddyville, Oskaloosa; Marion Co.—Tracy; Monona Co.—Onawa, Whiting; Muscatine Co.—Muscatine, Nichols, Wilton Junction; Osceola Co.—Ocheyedan; Page Co.—Shenandoah; Palo Alto Co.—Ruthven; Plymouth Co.—Merrill, Remsen, Westfield; Pocahontas Co.—Pocahontas; Polk Co.—Farrar, Des Moines; Pottawattamie Co.—Council Bluffs, Oakland, Taylor; Sac Co.—Lake View, Early; Scott Co.—Davenport, Le Claire; Sioux Co.—Alton, Chatsworth; Story Co.—Ames; Taylor Co.—New Market; Wapello Co.—Ottumwa; Washington Co.—Washington; Winnebago Co.—Lake Mills, Leland; Woodbury Co.—Sergeant Bluff, Sioux City, Sloan; Wright Co.—Cornelia, Dows, Galt.

**LARVAL RECORDS:** 148 collections; April (1.2), May (18.0), June (38.4), July (26.3), Aug. (37.5), Sept. (52.0).

**LARVAL HABITATS:** Collections were taken from waters of permanent, intermittent, or temporary natures; in ponds, stream bed pools, rain pools, pasture potholes, marshes, and flooded areas. They were also taken, in fewer numbers, from slow-flowing streams, artificial receptacles, and sewers.

**ASSOCIATED LARVAE:** *A. punctipennis* (62), *C. salinarius* (38), *C. apicalis* (35), *T. inornata* (35), *A. vexans* (31), *A. quadrimaculatus* (28), *U. sapphirina* (17).

**ADULT RECORDS:** May (1), June (4), July (2), Aug. (1), Sept. (5). In the field, biting females were usually encountered after dark and in a few instances were quite annoying.

### 32. *Culex restuans* Theobald

This species is also known in the literature as *C. territans* Walk.

**LOCALITIES:** Allamakee Co.—Harper's Ferry; Audubon Co.—Audubon; Blackhawk Co.—Waterloo; Boone Co.—Boone, Fraser, Ogden; Buena Vista Co.—Marathon; Carroll Co.—Coon Rapids; Cass Co.—Atlantic; Cerro Gordo Co.—Mason City, Ventura; Chickasaw Co.—New Hampton; Clarke Co.—Osceola; Clinton Co.—Follettes; Crawford Co.—Manilla; Dallas Co.—Perry; Davis Co.—Drakesville, Lake Wapello State Park; Des Moines Co.—Burlington; Dubuque Co.—Dubuque; Franklin Co.—Hampton; Greene Co.—Grand Junction, Jefferson, Scranton; Hamilton Co.—Jewell; Hancock Co.—Britt; Henry Co.—Mt. Pleasant; Iowa Co.—Homestead; Jones Co.—Anamosa; Keokuk Co.—Hedrick, Sigourney; Louisa Co.—Grand View; Lucas Co.—Lucas; Lyon Co.—Granite, Lester; Mahaska Co.—Eddyville; Marion Co.—Knoxville, Red Rock, Tracy; Monona Co.—Onawa; Monroe Co.—Albia; Muscatine Co.—Muscatine, Nichols, Wilton Junction; Palo Alto Co.—Ruthven; Plymouth Co.—Remsen, Westfield; Polk Co.—Des Moines; Sac Co.—Lake View; Scott Co.—Davenport, Montpelier; Story Co.—Ames; Tama Co.—Tama; Van Buren Co.—Farmington; Wapello Co.—Ottumwa; Warren Co.—Norwalk; Washington Co.—Washington; Winnebago Co.—Forrest City; Woodbury Co.—Sergeant Bluff, Sioux City.

**LARVAL RECORDS:** 69 collections; May (13.5), June (15.6), July (17.0), Aug. (62.5), Sept. (10.9).

**LARVAL HABITATS:** Pond margins, stream bed pools, summer rain pools, and marshes most frequently yielded larval collections. The waters of these habitats were generally permanent or of an evanescent nature. In a few instances larvae were collected from temporary bodies of water, artificial receptacles, and sewers.

**ASSOCIATED LARVAE:** *A. punctipennis* (31), *C. salinarius* (28), *C. tarsalis* (27), *A. vexans* (20), *C. pipiens* (14), *T. inornata* (13), *C. apicalis* (13).

### 33. *Culex salinarius* Coquillett

**LOCALITIES:** Allamakee Co.—Lansing; Blackhawk Co.—Waterloo; Bremer Co.—Waverly; Buena Vista Co.—Marathon; Butler Co.—Shell

Rock; Carroll Co.—Coon Rapids; Cerro Gordo Co.—Mason City; Clarke Co.—Osceola; Clay Co.—Dickens; Clinton Co.—Clinton, De Witt, Follett's; Crawford Co.—Astor; Davis Co.—Drakesville, Floris; Delaware Co.—Delhi; Des Moines Co.—Burlington; Dickinson Co.—Lake Park; Dubuque Co.—Dubuque; Greene Co.—Jefferson, Scranton; Hancock Co.—Britt, Crystal Lake; Henry Co.—Mt. Pleasant; Jackson Co.—Sabula; Jones Co.—Anamosa, Wyoming; Keokuk Co.—Sigourney; Lee Co.—Keokuk, Montrose; Louisa Co.—Grand Junction, Fruitland; Mahaska Co.—Eddyville; Marion Co.—Red Rock; Monona Co.—Onawa; Muscatine Co.—Muscatine, Nichols, Wilton Junction; Osceola Co.—Ocheyedan; Palo Alto Co.—Ruthven; Polk Co.—Des Moines; Pottawattamie Co.—Council Bluffs; Sac Co.—Lake View; Scott Co.—Davenport; Story Co.—Ames; Wapello Co.—Ottumwa; Warren Co.—Norwalk; Woodbury Co.—Brown's Lake, Sergeant Bluff, Sioux City; Wright Co.—Cornelia.

**LARVAL RECORDS:** 67 collections; May (11.3), June (17.5), July (7.6), Aug. (12.2), Sept. (27.4).

**LARVAL HABITATS:** Waters of permanent or evanescent natures in ponds, marshes, summer rain ponds, and stream bed pools produced most of the larval collections. These habitats were more permanent in nature than those in which *C. tarsalis* and *C. restuans* were usually taken.

**ASSOCIATED LARVAE:** *A. punctipennis* (40), *C. tarsalis* (38), *C. restuans* (28), *T. inornata* (20), *C. pipiens* (16), *A. vexans* (16).

**ADULT RECORDS:** June (6), July (3), Aug. (1), Sept. (2).

### 34. *Culex pipiens* Linnaeus

**LOCALITIES:** Blackhawk Co.—Waterloo; Boone Co.—Boone, Fraser; Butler Co.—Clarksville; Cass Co.—Atlantic; Clay Co.—Dickens; Clinton Co.—Clinton; Crawford Co.—Astor; Delaware Co.—Delhi; Des Moines Co.—Burlington; Dubuque Co.—Dubuque; Greene Co.—Grand Junction; Jackson Co.—Bellevue; Johnson Co.—Lone Tree; Keokuk Co.—Hedrick; Lee Co.—Keokuk, Montrose; Lyon Co.—Lester; Marion Co.—Knoxville; Monona Co.—Onawa; Muscatine Co.—Muscatine, Wilton Junction; Polk Co.—Des Moines; Sac Co.—Early; Scott Co.—Davenport, LeClaire; Story Co.—Ames; Woodbury Co.—Sioux City.

**LARVAL RECORDS:** 30 collections; May (2.2), June (5.2), July (5.9), Aug. (12.2), Sept. (16.4).

**LARVAL HABITATS:** Collections were taken most frequently from ponds and rain pools situated in city dumps. Artificial receptacles and open sewers produced several collections.

**ASSOCIATED LARVAE:** *A. punctipennis* (18), *C. salinarius* (16), *C. tarsalis* (15), *C. restuans* (14).

### 35. *Culex erraticus* D. and K.

**LOCALITIES:** Blackhawk Co.—Waterloo; Boone Co.—Fraser; Clarke Co.—Osceola; Clinton Co.—Follett's; Crawford Co.—Astor; Davis Co.—Drakesville; Delaware Co.—Delhi; Des Moines Co.—Burlington; Dubuque Co.—Dubuque; Jackson Co.—Green Island, Sabula; Lee Co.—Keokuk,

Sandusky; Louisa Co.—Fruitland, Oakville, Wapello; Lucas Co.—Lucas; Muscatine Co.—Muscatine; Scott Co.—LeClaire; Warren Co.—Lake Ahquabi; Woodbury Co.—Sergeant Bluff.

LARVAL RECORDS: 27 collections; June (0.6), July (6.8), Aug. (37.5), Sept. (20.6).

LARVAL HABITATS: The habitats of *C. erraticus* resembled closely those of *A. quadrimaculatus*. The collections were from permanent ponds supporting growths of aquatic floating plants. These ponds were usually in shaded areas.

ASSOCIATED MOSQUITO LARVAE: *A. quadrimaculatus* (18), *A. punctipennis* (18), *C. apicalis* (12), *C. tarsalis* (10).

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# ACTIVATED CARBON FROM CERTAIN AGRICULTURAL WASTES

T. R. McELHINNEY, B. M. BECKER, AND P. BURKE JACOBS

*From Agricultural By-Products Laboratory, Ames, Iowa<sup>1</sup>*

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Charcoal, which is obtained from the destructive distillation of agricultural wastes in yields of 20 to 30 per cent or more of various original dry raw materials (7), constitutes under existing markets more than 60 per cent of the potentially salable products of this process, exclusive of gas. The hardwood distillation plants in this country, until recently, depended for their profits largely upon the sale of such condensable volatile products as were rather easily refined and, except where it was used by an affiliated industry, marketed their charcoal largely as a low-cost fuel or for similar uses. In recent years, the market for the volatile products has been largely lost to similar or equal products of synthetic or fermentation origin which, in many cases, are produced in a purer form and at a lower cost than those from the destructive distillation process. As a result of the existing economic situation, and such loss of markets, the number of wood distillation plants operating in the United States has decreased in recent years to about one-half the number operating in 1925 (9, 17). Since the yields of all products from the destructive distillation of agricultural wastes are at best only equal to, and usually somewhat lower than, the yields of similar products obtained from hardwood (7, 10), it is apparent that successful utilization of a part of the large amounts of annually accumulated farm wastes by this process necessitates the discovery of new and more lucrative markets for the recoverable products. Obviously, since the volatile products are of lessening value, the finding of satisfactory markets for the charcoal would be an important factor in establishing destructive distillation as a successful agricultural waste industry.

The charcoal obtained from ligno-cellulosic raw material by the destructive distillation process exhibits great variation in chemical composition and properties, depending upon the method and the conditions of production as well as upon the raw material from which it is produced. Proximate analyses of various charcoals, as shown in Table 1, illustrate this variation. Analyses of charcoals produced from the same raw materials but with increasing distillation temperatures show decreasing amounts of volatile matter while the net fixed carbon remains practically constant (10). Charcoals produced at low temperatures are more apt to be contaminated by tarry matter than those produced at higher temperatures. The physical properties are also quite different. Aside from differ-

<sup>1</sup>Established by the Bureau of Agricultural Chemistry and Engineering, U. S. Department of Agriculture, in co-operation with Iowa State College.

TABLE 1

PROXIMATE ANALYSIS AND HEATING VALUE OF RAW REPORT CHARCOALS FROM HIGH AND LOW-TEMPERATURE DISTILLATIONS OF AGRICULTURAL WASTES

Source	VOLATILE MATTER PERCENTAGE		FIXED CARBON PERCENTAGE		ASH PERCENTAGE		HEAT VALUE B.T.U./lb.
	High Temp.* <i>T</i> <sub>1</sub>	Low Temp. <sup>†</sup> <i>T</i> <sub>2</sub>	High Temp.	Low Temp.	High Temp.	Low Temp.	
Straw .....	9.0	30.0	61.0	49.0	30.0	21.0	
Corncobs .....	8.0	17.0	86.0	79.0	6.0	4.0	13,500
Oathulls .....	8.0	15.0	68.0	67.0	24.0	18.0	10,560
Cornstalks .....	12.0	25.0	64.0	58.0	26.0	17.0	
Peanut hulls ..	10.0	14.0	82.0	80.0	8.0	6.0	13,000
Rice hulls .....	4.0	8.0	41.0	44.0	55.0	48.0	
Black walnut shells .....	5.0	20.0	92.0	78.0	3.0	1.5	
Cottonseed hulls .....	10.0	40.0	81.0	53.0	9.0	7.0	13,100
Pecan shells ..	5.0	20.0	89.5	77.5	5.5	2.5	12,425
Tobacco stems ..	20.0	44.0	52.7	39.3	27.3	16.7	

\* Above 550°C.

† Below 550°C.

ences in size, shape, and density of the particles, factors largely dependent upon the original condition of the material distilled, the charcoal may vary from a dark brown, tough material produced at low temperature to a gray-black, brittle material from a high-temperature distillation. A suitable charcoal must therefore be chosen for any proposed use. The present ordinary use for charcoal is limited.

These charcoals have heating values approximating bituminous coal (13,000-14,000 BTU/lb.) as can be seen in Table 1, but the sale or use of charcoal for fuel appears to be ordinarily uneconomical, unless in a higher-priced briquetted form for special uses, due to the relatively low cost or greater adaptability of coal, in most areas.

Some use has been made of the charcoal from oathulls and straw as a pigment in paints, and in the past, at least one commercial firm marketed such paints which were claimed to be particularly effective as metal protectives (7), although such claims have not yet been wholly proven. Other possible uses for farm waste charcoals include the manufacture of charcoal iron, casehardened steel, calcium carbide, gun powder, and activated carbon (16). Of these possible uses, activated carbon offers perhaps the most favorable potential outlet, due to the quantities which might be utilized. At present, over 1,000 waterworks plants in the United States are using activated carbon for the removal of taste and odor in their waters (1), while large quantities of activated carbon of other types are also used in solvent recovery, gas recovery, air purification, etc. It is probable that a properly developed market would offer opportunity for consumption of large quantities of destructive distillation charcoal in the form of activated carbon, provided such carbon can be produced economically.

The need for activated carbon for use in gas masks during the war of 1917-18 necessitated a study of methods of producing it. Methods were developed by the War Department (6) which produced acceptable activated carbons from dense raw materials such as coconut shells, anthracite coal, lignite, and suitable briquetted carbon dusts in general. Raw materials now used for commercial activated carbons include principally lignite, paper mill waste liquors, and wood charcoal. Many processes have been patented for the preparation of activated carbons (5, 2, 11, 13, 18). These processes have been classified by Mantell (8) and by Ray (12).

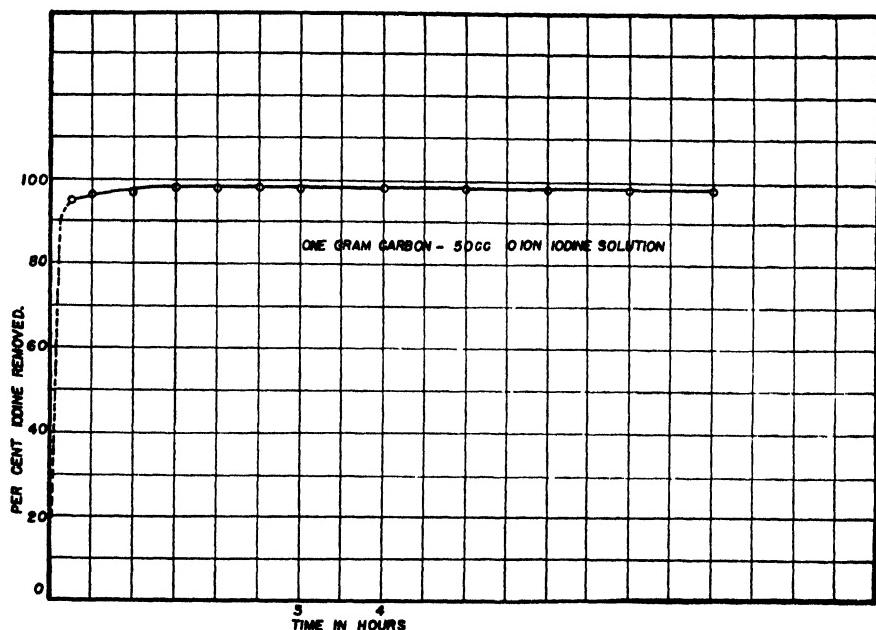


Fig. 1. Rate of removal of iodine by activated pecan shell carbon.

Preliminary laboratory tests of various raw retort charcoals produced from farm waste materials for effectiveness in the decolorization of sugar, iodine, and methylene blue solutions showed in occasional instances some decolorizing power against the iodine and the methylene blue, but none against the sugar solutions. Cold water extractions showed the presence of extractable tar bodies in practically every sample of charcoal, in quantities which varied somewhat inversely with the temperatures of distillation at which the carbons were produced. A preliminary test showed that such charcoals, if heated during passage of a current of steam, could be relatively freed from this tarry matter and would show, thereafter, considerable decolorizing power. Steam activating experiments were therefore run to determine the extent of treatment necessary for certain representative types of material.

### EXPERIMENTAL

The steam activation method of Chaney (4, 5) was selected as the one most easily adaptable to laboratory experimentation. Since the activation process has already been studied so intensively and since the conditions of treatment will depend upon the material selected for activation, this experimentation was not undertaken particularly with the idea of determining exact conditions for activating each specific material, but only to determine the general applicability of the process to carbons produced from agricultural wastes.

**BATCH ACTIVATION.** The apparatus used in the laboratory batch experiments in this work is shown in Figure 2. It consisted of an electrically-heated steam generator, a superheater (a coil of 3/16-inch copper tubing heated by a Bunsen burner), and a 15-inch section of  $\frac{7}{8}$ -inch internal

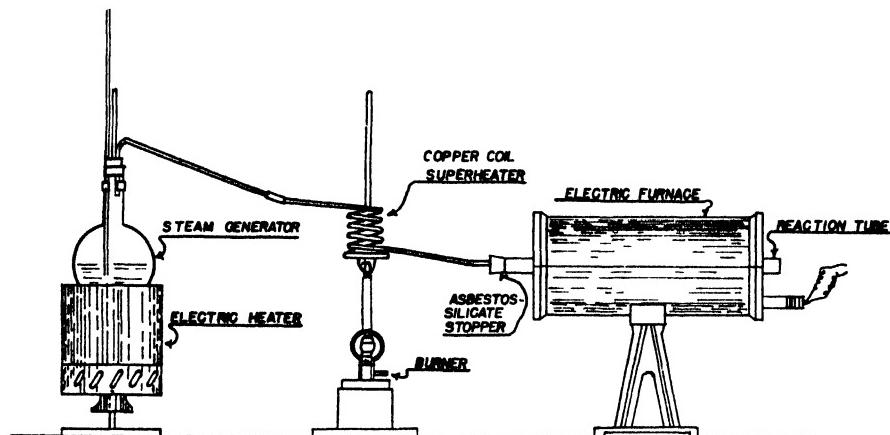


Fig. 2. Laboratory activating equipment.

diameter fused-silica combustion tube mounted in a heavy-duty electric combustion furnace. A plug of nichrome wire was inserted in the end of the reaction tube to prevent carbon from being blown out with the steam. The connection between the reaction and steam tubes was made with an asbestos stopper sealed in place with a paste made of sodium silicate and borax. In some of the preliminary work, instead of a superheater, pieces of broken silica tubing were used at the entrance of the reaction tube, but the inconsistency of the results indicated that this was not a dependable means of superheating the steam.

In making a run, the furnace was first brought to the desired temperature, as indicated by a pyrometer, whereupon the reaction tube, containing a weighed charge of carbon, was inserted and connected to the steam generator. The carbon was moistened, after weighing, to prevent burning during the short interval necessary to make and seal the steam connection. At completion of the arbitrarily predetermined activation treatment, the contents of the reaction tube were quickly emptied into

distilled water, to stop air oxidation, the water drained away, the carbon dried to constant weight at 110° C., and its relative activity determined. The exact temperature of the steam as used was not determined, but the superheating coil was kept as near as possible at a dull red heat at all times. The steam was supplied at as even a rate as is possible with a definite setting of an electric heating unit, without checking the voltage from day to day. Experiments were made to determine approximately the time, temperature, and amount of steam required to accomplish some degree of activation in various charcoals.

**CONTINUOUS ACTIVATION.** Following the establishment of approximate activating conditions and degree of activation in the preliminary work, a continuous activating furnace was built. This apparatus, Figure III, consisted of a section of 2-inch steel pipe, slightly over 4 feet in length, which contained an internal screw conveyor. A motor drive was constructed to turn the screw at such a rate that the carbon required 30

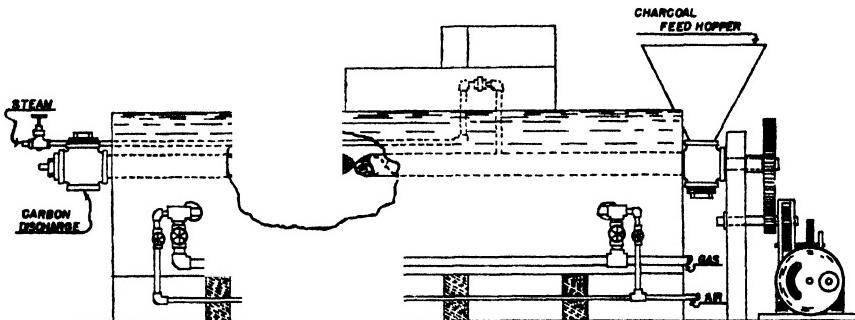


Fig. 3. Continuous activating furnace.

minutes for passage through the equipment, a hand crank having been used in early trials made to determine the optimum time. This unit was mounted in a suitable brick-lined furnace. Heat was supplied by two gas burners of appropriate size constructed from pipe fittings, and mounted obliquely to secure uniform heating of the reaction tube. The firebox was filled with broken pieces of refractory material to a level above the burners to induce surface combustion. Compressed air was supplied to the furnace. A hopper for continuously charging the material was attached at one end of the activator. The hot discharged material was allowed to fall into a metal container which was carefully shielded so that the gaseous reaction products effectively smothered the burning carbon, thus preventing oxidation. Temperatures were determined by means of thermocouples mounted in the furnace so as to rest on top of the 2-inch pipe. On the average this apparatus produced one pound of activated carbon per hour from dense charcoals, although this amount varied inversely with the weight loss during activation. Steam was controlled only insofar that the rate of flow was held just below the point where carbon was blown out of the reaction tube.

## METHODS OF TESTING

**DECOLORIZING POWER.** A search of the literature showed that there was no dependable general test for evaluation of activated carbons at the time of conducting these experiments and that, generally, carbons could be compared in their effectiveness only under actual conditions of use. Therefore, for these studies, the following arbitrary procedure was adopted as a simple and easy comparative test for determining the relative decolorizing power:

One gram of oven-dry activated carbon, ground to pass a 200-mesh screen, was placed in a flask containing enough 0.10 N iodine solution (made up with potassium iodide) to give approximately a 10 per cent excess above the probable total adsorption, and kept in the dark 2½ hours with occasional shaking, at the end of which time the carbon was filtered out and washed once with 20 to 25 cc. of water, and the filtrate titrated with standard sodium thiosulfate solution. Results are expressed in cc.'s of iodine adsorbed and not removed by washing per gram of carbon.

In working out this method of testing, it was found that the amount of iodine adsorbed varied with the size and physical condition of the carbon particles, with the length and temperature of the exposure, and,

TABLE 2  
PRELIMINARY ACTIVATION OF VARIOUS CHARCOALS IN BATCH EQUIPMENT WITHOUT SUPERHEATER

Run No.	Source of Charcoal	Time of Run Minutes	Temperature °C.	Steam Rate	Wt. Loss Percentage	N/10 I <sub>2</sub> Adsorbed cc.
0 . . . .	Oathulls	Not activated	.....	.....	.....	16.50
1 . . . .	"	5	680	.....	.....	16.23
2 . . . .	"	10	635	.....	.....	17.10
3 . . . .	"	60	620	.....	.....	18.41
4 . . . .	"	30	650	.....	.....	18.74
5 . . . .	"	30	760	.....	.....	21.58
6 . . . .	"	30	870	.....	.....	37.06
7 . . . .	"	45	816	.....	.....	29.52
8 . . . .	"	45	827	.....	.....	31.58
9 . . . .	"	45	843	.....	.....	34.82
10 . . . .	"	45	857	.....	.....	41.65
11 . . . .	"	45	871	.....	.....	42.44
12 . . . .	"	10	871	.....	.....	26.30
13 . . . .	Corncobs, low temp.	30	871	.....	.....	41.20
14 . . . .	Straw, low temp.	30	871	.....	.....	31.50
15 . . . .	Rice hulls	30	871	.....	.....	20.45
16 . . . .	Pecan shells	20	627	.....	.....	26.00
17 . . . .	" "	20	900	.....	.....	48.39
18 . . . .	" "	30	950	.....	.....	49.20
19 . . . .	" "	Not activated	blank	.....	.....	24.00
20* . . . .	Pecan shells	45	950	.....	.....	48.15
21 . . . .	" "	30	950	Low	73.5	49.45
22 . . . .	" "	30	950	Moderate	47.0	49.20
23 . . . .	" "	30	950	Very high	35.7	37.10

\* Raw pecan shells, carbonized during activation.

to some extent, with the pH of the mixture. Adsorption is ordinarily very rapid in the first few minutes, proceeds at a decreasing rate for several hours and finally virtually ceases as equilibrium is established. Figure I shows a typical adsorption curve for an activated pecan shell carbon.

**ODOR TESTS.** A few tests were made of the effectiveness of these carbons in the removal of odor in well water, using the "Threshold" method of odor determination as developed by Spaulding (14, 15) and Baylis (3).

### RESULTS

Results on preliminary work on a laboratory scale are shown in Table 2. Runs 1 to 3, inclusive, shown therein, were made at temperatures between 600° and 700° C. and showed little or no improvement over untreated charcoal. Runs 4 to 6 show the effect of increasing temperatures. Runs 7 to 12 show the effect of increased temperatures during a longer period of time, run 12 being included to definitely show the effect of time. Runs 13 to 20 were made under arbitrarily chosen conditions to obtain preliminary information on carbons from various raw materials. It will be noted that raw pecan shells can be carbonized and simultaneously activated by this method, but it is questionable whether such a procedure would be economic without recovery of by-products, in contrast to preliminary destructive distillation of the shells. Runs 21 to 23 were made in an attempt to evaluate the effect of steam rate. The results obtained were the reverse of what would be expected and are thought to be due to a lowering of the temperature in the reaction tube, caused by the quality of the steam used. These results indicated the need of a superheater.

Results with the use of superheated steam are shown in Table 3. Runs

TABLE 3  
ACTIVATION OF VARIOUS CHARCOALS IN BATCH EQUIPMENT WITH SUPERHEATER

Run No.	Source of Carbon	Time Min.	Temp. °C.	Steam gm./gm. Carbon	Wt. Loss on Raw Carbon Percentage	N/10 L Adsorbed cc.
24 ....	Pecan shells low temp.dist.	30	900	11.68	63.40	48.79
25 ....	"	30	915	10.30	56.00	48.75
26 ....	"	30	950	16.60	79.00	48.88
27 ....	"	30	950	7.20	87.20	49.23
28 ....	Pecan shells high temp.	30	950	9.34	70.0	49.28
29 ....	Corncobs, low temp.	10	950	5.20	38.70	49.00
30 ....	"	12	950	6.84	46.00	49.17
31 ....	"	15	950	7.80	56.25	49.36
32 ....	"	20	950	Practically all burned up.		No test.
33 ....	Cornstalk* pressboard	30	950	7.13	77.00	39.70
34 ....	Oathulls	30	950	16.10	74.00	36.25

\* Cornstalk pressboard which had been destructively distilled to determine relative yields of by-products from whole and compressed (briquetted) cornstalks.

24 to 27, made on pecan shell carbon from a low temperature distillation, indicate that relative iodine adsorption apparently increases with increase of processing temperature and weight loss of charcoal during treatment, but that this increase in adsorption is not directly proportional to either. Weight loss, generally, increases with an increase in steam consumption, although run 27 is an exception to this rule, possibly explained either by the fact that the rate of steam flow was so low as to permit burning or, more probably, that the low rate of passage through the superheater permitted the steam to become hotter than usual. The data from this table show that relatively soft corncob carbon becomes as active as the denser pecan shell carbon with considerably shorter time of treatment and with lower steam consumption and weight loss. This may be explained by the fact that corncob charcoal has a lower apparent density and, therefore, a greater adsorption area per unit weight than pecan shell

TABLE 4

## COMPARISON OF ADSORPTION ABILITIES OF THE BEST CARBONS FROM BATCH SET-UP WITH COMMERCIAL CARBONS

Run No.	Source of Carbon	Time of Run Minutes	Temperature °C.	N/10 L Adsorbed cc.
11 .....	Oathulls	45	871	42.44
13 .....	Corncobs	30	871	41.20
14 .....	Straw	30	871	31.50
15 .....	Rice hulls	30	871	20.45
21 .....	Pecan shells	30	950	49.45
27 .....	Pecan shells	30	950	39.70
33 .....	Cornstalk pressboard	30	950	39.70
31 .....	Corncobs	15	950	49.36
34 .....	Oathulls	30	950	36.25
"Commercial carbon No. 1" .....				43.00
"Commercial carbon No. 2" .....				46.90
"Commercial carbon No. 3" .....				42.05

carbon. Cornstalk and oathull charcoals are less satisfactory for activation, due to their high ash contents.

Table 4 gives a comparison of the best agricultural waste carbons, produced on the laboratory scale, with certain commercial carbons widely used in water treatment. On the basis of iodine adsorption alone, it is shown that oathull, corncob, pecan shell, and cornstalk charcoals can be converted into activated carbons comparable to commercial products from other sources.

Table 5 shows the production data and iodine adsorption values for pecan shell carbons activated in the continuous activating apparatus described above. Runs 35 to 48, made without the use of superheated steam, show adsorption values somewhat lower than those obtained from carbons made in the batch furnace. The remaining runs, using a superheater, produced carbons generally better than those produced in the batch fur-

TABLE 5  
ACTIVATION OF PECAN SHELL CARBON IN CONTINUOUS ACTIVATING FURNACE

Run No.	Time of Passage Minutes	Temperature °C.	Wt. Loss Percentage	Remarks	N/10 L Adsorbed cc.
35 .....	30	625	.....	No super-heater	34.15
36 .....	30	760	.....	High pressure (100 lb.)	42.10
37 .....	5	843	19.1	steam only	37.00
38 .....	30	850	45.0	"	39.60
39 .....	15	890	17.1	"	34.30
40 .....	30	760	.....	"	48.00
41 .....	30	840	.....	"	44.60
42 .....	30	840	.....	"	42.30
43 .....	30	840	.....	"	47.80
44 .....	30	800	.....	"	47.40
45 .....	30	820	.....	"	47.57
46 .....	30	850	.....	"	47.00
47 .....	30	810	.....	"	37.10
48 .....	30	820	.....	"	36.80
49 .....	30	871	45.4	Superheater used	52.20
50 .....	30	871	42.5	"	51.66
51 .....	30	871	42.0	.....	51.13
52 .....	30	800	62.4	.....	51.04
53 .....	30	852	63.6	.....	52.21
54 .....	30	888	67.9	.....	52.43
55 .....	30	907	61.6	.....	52.46
56 .....	30	880	72.0	.....	52.21
57 .....	30	816	46.2	.....	46.36
58 .....	30	849	36.4	.....	47.62
59 .....	30	871	36.2	.....	49.31
60 .....	30	843	37.4	.....	50.79
61 .....	30	883	34.9	.....	50.54
62 .....	30	883	38.9	.....	49.59
63 .....	30	940	41.7	.....	50.65
64 .....	30	992	68.2	.....	52.38
65 .....	30	960	65.9	.....	52.48
66 .....	30	920	51.8	.....	51.79
67 .....	30	1000	60.2	.....	50.84
68 .....	30	980	68.6	.....	50.71
69 .....	30	980	70.0	.....	50.84
70 .....	30	980	60.1	.....	49.73
71 .....	30	955	45.2	.....	49.20
72 .....	30	950	48.6	.....	50.50

nace. It is apparent that the data obtained were not sufficient to explain the results in this series of runs. However, the results obtained indicated that the continuous furnace would be quite practical for this work when provided with suitable controls. It can be seen from Table 5 that it would be economical to sacrifice some adsorptive power in order to obtain a high yield, since conditions causing greater than a 40 per cent loss in weight of original charcoal do not increase the adsorption sufficiently to

warrant the additional loss of material. Although optimum conditions were not fully established, runs 49 to 51, and 58 to 63, inclusive, indicate that it is possible to obtain, relatively, very active carbon (based on iodine adsorption) with a comparatively low weight loss. This would predicate the possibility of manufacturing a commercial carbon for a single or limited use, with decolorizing value somewhat below present commercial standards, at a possible saving in cost.

A test was made to determine the effect of the size of charcoal particles prior to activation upon the activity of the finished carbon. Charcoal was ground so as to have a maximum particle size of  $\frac{1}{4}$  inch and activated, without screening, at  $970^{\circ}$  C. for 30 minutes. The activated carbon was then classified for particle size: (1) material passing a 200-mesh screen, (2) material passing a 1-mm. screen and retained on 200-mesh, and (3) material retained on a 1-mm. screen. A portion of each sample (ground to 200-mesh), both untreated and acid-washed to pH 6, was tested for iodine adsorption with results as follows:

Portion	cc. 0.10 N I <sub>2</sub> adsorbed (untreated)	cc. 0.10 N I <sub>2</sub> adsorbed (acid-washed)
1 .....	41.05	46.68
2 .....	47.73	48.33
3 .....	48.85	48.79

The lower adsorption shown by the finer unwashed portion is probably due to the relatively higher ash content, the result of overburning of the smaller particles by the heat required to activate the larger particles. An acid wash applied to such a material somewhat increased its adsorption, the increase amounting to 11 per cent for the finer portion, as shown above. Since the finer material will probably always be overtreated under conditions which will activate the larger particles, it would appear desirable to grade the carbon to size prior to treatment and then to vary the operating conditions to suit the particle size. Where a fine product is desired, considerable economy in heat and time might be effected by grinding to the required degree of fineness before activating, with suitable processing adjustments.

During the course of this study information was received<sup>2</sup> that under certain conditions carbon reacts with potassium iodide solution to form hydriodic acid and that it is advisable to make adsorption tests on water solutions of iodine with no potassium iodide present. The procedure used in this laboratory follows:

Twenty milligrams of the carbon to be tested is added to 200 cc. of an iodine solution containing approximately 100 p.p.m. iodine. The flask is tightly stoppered, shaken fifteen minutes, and the contents promptly filtered through filter paper. The first 50 cc. of filtrate is discarded and a 50 cc. portion of the remain-

<sup>2</sup> Private communication.

der, to which 5 cc. of 10 per cent KI solution is added, is titrated with 0.01 N sodium thiosulfate. A blank, to which no carbon is added, is run at the same time. Calculations are based on the iodine remaining in the solution, as shown by the titration.

For handling such small quantities of carbon, it was found convenient to make up a water suspension of the carbon. For these tests, 1.2 g. of the carbon was placed in a 300-cc. volumetric flask which was then filled to the mark with distilled water. Five cc. of the suspension was equivalent to 20 milligrams of carbon.

The tests using iodine alone show a greater variation in results than those made using iodine in potassium iodide solution, but they are in the same order in each case, as indicated in Table 6.

A few comparisons were made between laboratory-prepared pecan shell carbon and a commercial carbon as to their effectiveness in odor

TABLE 6  
RELATION OF I<sub>2</sub> AND I<sub>2</sub>-KI TESTS AS A MEASURE OF ADSORPTION CAPACITY

Run No.	SOURCE OF MATERIAL	I <sub>2</sub> TEST		I <sub>2</sub> -KI TEST	
				I <sub>2</sub> Adsorbed	
		mg. I <sub>2</sub> Adsorbed per Gram Carbon	cc.	mg. per Gram Carbon	cc.
60	Pecan shells, pH 9 .....	475*	.....	.....	.....
60	Pecan shells, pH 5 .....	714*	.....	.....	.....
60	Pecan shells, unwashed .....	497*	50.79	645	.....
	Commercial carbon No. 3	446*	42.05	534	.....
	Commercial carbon No. 3	421*	43.00	546	.....
60	Pecan shells, pH 9 .....	840†	.....	.....	.....
60	Pecan shells, pH 5 .....	853†	.....	.....	.....
31	Corncobs .....	823†	49.36	627	.....
	Commercial carbon No. 1	531†	43.00	546	.....
	Commercial carbon No. 4	411†	.....	.....	.....

\* I<sub>2</sub> concentration = 90.6 parts per million.

† I<sub>2</sub> concentration = 113.0 parts per million.

removal of drinking water in actual treatment at the works. The water treated was raw well water, at the Ames, Iowa, Waterworks Well No. 2. This water was contaminated with seepage from an old gas plant tar dump and has a particularly noticeable and rather objectionable odor of phenol. Dosages of 10 p.p.m. pecan shell carbon in the settling basins reduced the "Threshold Odor Number" (14, 15, 3) to 2 while the same treatment with equal amounts of "Commercial Carbon No. 1" reduced it only to 4. This work was discontinued before conclusive tests were completed, but the results were seemingly satisfactory.

A tentative calculation as to the economies of producing activated carbon from pecan shells can be made, provided certain assumptions are adopted.

Assuming a 27 per cent yield of primary charcoal (average, reference (7)) from pecan shells, the shells assumed at \$2.25 per ton f.o.b. distilla-

tion plant, and assuming further that the cost of producing the primary charcoal by destructive distillation was offset by the value of the condensable vaporized products obtained therefrom, then the raw charcoal would cost about 0.4 cents per pound. A loss of 50 per cent in weight, (Table 5) in activation, would raise the cost of the raw material to about 0.8 cents per pound of activated carbon produced. Assuming an additional "activating cost" of 0.5 cents per pound, for labor, steam, and heat, plus 0.7 cents for overhead, sales, and miscellaneous, a bulk price of 2 cents per pound, f.o.b. plant, or \$40.00 per ton, might be predicated. Such costs might be commercially competitive.

#### ODOR REMOVAL VALUE OF PECAN CARBON

The following tests comparing pecan shell carbon prepared in this laboratory with commercial carbons were made by a commercial water company.<sup>3</sup> Their report, in part, follows, with the commercial carbons identified as in the tables given above.

1. Specific gravity.  
Commercial carbon No. 2—0.406  
Commercial carbon No. 1—0.273  
Pecan shell carbon —0.465
2. Iodine adsorption (28 mg. I, plus 20 mg. carbon)  
Commercial carbon No. 2—43 per cent removal  
Commercial carbon No. 1—37 per cent removal  
Pecan shell carbon —38 per cent removal
3. Odor reduction tests using aldehol  
Procedure: 1 cc. aldehol in 250 cc. water. 25 cc. of this added to 4,000 cc. water to give a dilution of 1 to 40,000 aldehol, a very strong concentration with a pungent odor of noticeable intensity.

Aldehol solution of this concentration was placed in three 1-gallon bottles. 72 milligrams of "Commercial carbon No. 1" was placed in one bottle, 72 milligrams of "Commercial carbon No. 2" was placed in another, and 72 milligrams of pecan shell carbon in the third. This dose is equivalent to about 150 pounds per million gallons or slightly more than a grain per gallon. The order of effectiveness in removing this odor was as follows:

1. Pecan shell carbon
2. "Commercial carbon No. 2"
3. "Commercial carbon No. 1"

The contact period was 3 hours.

#### CONCLUSIONS

1. Activated carbons can be produced from certain agricultural waste materials, either directly or from charcoals resulting from destructive distillation.
2. Production of activated carbon from agricultural waste materials represents a potential outlet for utilizing large quantities of these materials, provided the economic phases are in satisfactory adjustment.
3. With proper control of time, temperature, steam, particle size, and pH of the finished products, the indications are that activated carbons

\*Private communication.

equal, or superior, to many of those now on the market could be produced from certain agricultural wastes, for specific purposes such as water treatment. The relative economics of commercial production of agricultural carbons must be studied in more detail before final conclusions can be drawn.

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# A LIST OF CHINESE MIRIDAE (HEMIPTERA) WITH KEYS TO SUBFAMILIES, TRIBES, GENERA AND SPECIES<sup>1</sup>

TSAI YU HSIAO<sup>2</sup>

From the Department of Zoology and Entomology, Iowa State College

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Our knowledge of the Family Miridae is fairly complete for the European and North American species largely owing to the contributions of Prof. O. M. Reuter of Finland and Dr. H. H. Knight of the United States. Of the Chinese species very little is known. The first description of a Chinese mirid, *Pachypeltis chinensis* was by V. Signoret in 1858. Since that time eighteen papers have appeared in which Miridae are described from China. Among these contributions two papers by Reuter, one by Lindberg, and one by Hsiao are entirely devoted to Chinese fauna and hence indispensable references for the study of the mirid-fauna of China.

Reuter (1903) in his "Capsidae Chinensis et Thibetanae" described four genera, ten species, and two varieties, among which six species have locality records as "Thibetia (Mou-Pin)." This is apparently a mistake as Mou-Pin should be referred to Szechuan province rather than Tibet. In his "Uebersicht der palaarktischen Stenodema-Arten," Reuter (1904) described four species from China. In 1906 he published the most important paper on Chinese Miridae "Capsidae in pro. Sz'tschwan China" in which he described seven genera, thirty-eight species, and nineteen varieties. These descriptions are based on the collections of G. Potanin and M. Beresowski. Some of the locality records are given in local pronunciation of small places which the writer has been unable to identify as to their exact location. However, one can follow the track of the collectors between known cities by the dates given, thus the provinces can be recognized if not the towns. In 1934 Lindberg described five species from the collection of the Swedish-Chinese scientific expedition to the northwestern provinces of China. The present writer (1941) has described eleven species and one variety. Besides these a few odd species were described by Bergroth, Distant, Jakovlef, Kiritshenko, Poppius, Reuter, and Walker, and their references will be found in the citations under each species.

These, together with some exotic species recognized in China by the writer, total 143 species and 32 varieties which are distributed in 60

<sup>1</sup>Taken from a thesis (No. 618) presented in partial fulfillment of the requirements for the degree of Doctor of Philosophy in the Graduate College of Iowa State College, June, 1941.

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genera and 7 subfamilies. About 60 per cent of the locality records are from Szechuan province, 18 per cent from Kansu province, 15 per cent from Mongolia and the remaining 7 per cent from other parts of China.

Zoogeographically, China is mainly Palearctic from the northern border to the Yangtze River, and Oriental for the southern part, while the Indo-Maylayan fauna extends northwards to some of the southwestern provinces. The provinces of Szechuan and Sikang serve as the centering point where these faunas meet. Hoffman (1935) divided Eastern Asia zoogeologically into nine subregions of which China embraces three, the Indo-Chinese, Manchurian, and Siberian subregions. In the present study there is not enough material available to recognize these divisions. However, it is interesting to notice that in the present study one Philippine species has been collected as far north as Peiping and a few Indian species collected inland as far as Szechuan province. As has been shown above, the greater part of China has not been well collected, and much work needs to be done before we can form a complete picture of the Chinese mirid-fauna.

The present study is an attempt to summarize our fragmentary knowledge of the Chinese Miridae and to arrange them systematically as our present knowledge permits. Synoptic keys to subfamilies, tribes, genera, and species are given for all the species of the family known to occur in China. Structural characters have been utilized as far as possible in preparing the keys. For measurements of body parts the following explanation is made: width of pronotum is the distance between the two posterior angles; the width of vertex is the shortest distance between the interior margins of the eyes unless otherwise indicated, and the width of eye is its transverse dimension as viewed from above. The Chinese locality records are arranged according to province. Those given only as "China" will be found enclosed within quotation marks.

#### GENERAL DISCUSSION

Early in 1831 C. W. Hahn, in his famous "Wanzenartigen Insecten," founded the family "Mirides," based upon Fabricius' genus *Miris*, the oldest genus of the family. Four years later H. Burmeister used the name "Capsini" for the family, and unfortunately this was followed by most of the early hemipterists. It was not until 1899 that G. W. Kirkaldy pointed out that according to the "rule of priority" Miridae should be used as the correct name of the family as it is based on both the oldest genus, *Miris*, and the oldest group name, "Mirides."

This is an extremely large family. In the palearctic region it includes nearly one-third of all the hemipterous species, and forms nearly one-fifth of all the species of Hemiptera and Homoptera listed in Oshanin's Katalog. They are mostly fragile insects with more or less soft integument. They are active, run fast, and fly freely.

The Miridae, being represented by both pagiopodous and trochalopodous types, are considered phylogenetically as relatively primitive Hemip-

tera. Latreille (1825) and others placed them close to Pyrrhocoridae on the basis that they are without ocelli. Considering other characters it is evident that this conclusion is arbitrary. Fieber (1851) placed the Miridae close to Cimicidae and Anthocoridae, and Kirschbaum (1855) first pointed out the relationship of Miridae with Anthocoridae on the basis of the development of a cuneus in the hemelytra. This opinion has been accepted by most of the hemipterists. Reuter (1910), in his monumental work, "Neue Beiträge zur Phylogenie und Systematik der Miriden," included the Miridae in the superfamily "Cimicoideae" together with Isometopidae, Termatophylidae, Microphysidae, Anthocoridae, and Cimicidae. As to the phylogenetic relationship among the subfamilies of Miridae, Knight (1923) pointed out that it does not present a linear series of development, but more of a progression upward in several directions, Phylinae being the most primitive group and Capsinae representing the highest development.

The mirids are to be found in varied situations depending upon the species. Some species inhabit low, marshy places while others prefer dry, sandy hills, but all are terrestrial. A majority of the species subsist exclusively on plant juices, but certain genera are known to be chiefly predaceous, preying upon soft-bodied insects. In certain species the predaceous habit is only partially developed. The greater number of the plant feeders are limited to a single host plant or to closely related species of plants. But there are a few species, such as *Lygus pratensis* Linn. and *Adelphocoris lineolatus* Goeze, which have a great range of food plants, and often become serious pests of cultivated crops.

Many species of Miridae exhibit pterygomorphism. Brachypterous and apterous forms occur throughout the family, and individuals of one species may exhibit different forms in regard to wing-development, having all gradations of brachypteryism. In some species only females are dimorphous, while in others both males and females are dimorphous. Accompanying the development of wings, there occurs in some species a corresponding variation of development of the thorax and consequently the dimorphism of the body-form. The genus *Mecomma* serves as an excellent illustration. In this genus male and female are usually so different that an inexperienced worker can scarcely free himself from the belief that they should belong to different species. In general, the male is more slender than the female and with narrower vertex and longer antennae.

Being mostly plant feeders, a number of species of mirids exhibit the phenomenon of protective coloration. Those inhabiting low vegetation, among roots of grasses, are generally dark, those living on leaves of grasses and trees are greenish, and those feeding on conifers, reddish or yellowish. Certain species of *Stenodema* imitate their host plants in coloration to such extent that they can change their color in different seasons to simulate host colors. Numerous species exhibit great variation of color patterns, and color varieties within the species are also frequent. Different sexes are often differently colored, the male usually darker than

the female. The individuals of over-wintering species usually become darker after hibernation.

Mimetic forms are numerous among the Miridae, especially the species of *Pilophorus* and *Myrmecoris* and their closely related genera. These species commonly mimic the appearance of ants in whose community they are often collected, and at first glance may be mistaken for ants. The nymphs of *Bothynotus pilosus* show a strong similarity to the plant lice on which they apparently subsist. The relationship between the antlike mirids and ants still remains an unexplained mystery.

A number of mirids, like many other hemipterous insects, may emit a kind of offensive odor. However, some species of *Apocremnus* have been reported to emit sweet odor, and some *Calocoris* have ostiolar odor like the scent of pears. The explanation of this phenomenon offers an interesting problem for research.

The family Miridae is characterized as follows: body medium or small, rarely large, longer than broad and broader than high, pubescent or glabrous. Head with juga not reaching apex of clypeus, ocelli absent. rostrum free, four-segmented, segment I thickened and as long as or longer than head; antennae four-segmented, segment I the thickest, segments III and IV usually more slender than II, rarely as thick or thicker. Hemelytra typically separated into clavus, corium, cuneus, and membrane, embolium sometimes discrete with corium, veins of membrane forming a major and a minor areole. Posterior coxae cardinate or sometimes rotatory, tarsi three-segmented, claws usually provided with arolia or pseudarolia or both at base, the presence or absence, size and form of these structures furnish the most reliable characters for separating the subfamilies.

This family was divided by Doctor Knight into nine subfamilies seven of which are known to occur in China, though the subfamily Cylapinae is likely to be found when more collection is made.

#### FAMILY MIRIDAE

##### Key to Subfamilies

1. Arolia absent, or present but bristle-like in form, pseudarolia absent or present and sometimes very prominent.....2  
Arolia prominent, always arising approximately at base between the claws, sometimes minute pseudarolia also present.....6
2. Prothorax simple, without an annuliform apical stricture, sometimes with a flattened apical area suggesting a collar but not separated off by a distinct incised line.....*Phyllinae*  
Prothorax with an apical stricture, sometimes obsolete above in the middle but forming a distinct collar.....3
3. Pseudarolia present, usually prominent.....5  
Pseudarolia absent, arolia bristle-like.....4
4. Claws slender and simple, evenly curved.....*Cylapinae*  
Claws angulatedly bent, usually cleft near base.....*Deraeocorinae*
5. Pseudarolia large or small, not connate with the claws; tarsal segment III linear, not thicker than the preceding.....*Dicyphinae*

- Pseudarolia large, broadly involving the claws; tarsal segment III incrassate, always thicker than the preceding.....*Bryocorinae*
6. Arolia converging at their apices.....*Orthotylinae*  
Arolia diverging at their apices.....7
7. Prothorax without distinct annuliform apical stricture, tarsal segments scarcely overlapping at joints and thus very flexible.....*Mirinae*  
Prothorax with an annuliform apical stricture extending over the sides and beneath, tarsal segments with tips overlapping at joints and thus practically inflexible .....
- Capstnae*

#### SUBFAMILY PHYLINAE

##### Key to Tribes

1. Pronotum with a more or less flattened apical area, lateral margins distinctly sinuate; abdomen distinctly constricted at base; pseudarolia not distinguished .....
- Allodapini*  
Pronotum simple, lateral margins straight or nearly so, rarely slightly sinuate; abdomen not constricted at base.....2
2. Body above usually shining; prosternal xyphus convex, immarginate or rarely slenderly marginate, pseudarolia minute and connate upon the inner angle of claws or wanting.....*Phylini*  
Body above usually opaque, prosternal xyphus depressed on its disk, its margins more or less elevated; pseudarolia distinct, extending beyond middle of claws.....*Oncotylini*

#### TRIBE PHYLINI

##### Key to Genera

1. Head wide, not less than four-fifths of width of pronotum at base, legs saltatorial .....
- Chlamydatus*  
Head medium or small, less than four-fifths of width of pronotum at base, legs normal.....2
2. Clypeus depressed, broad, confluent or obsoletely discrete with frons; vertex marginate .....
- Sthenarus*  
Clypeus compressed, prominent, generally discrete with frons by an impression at base; vertex immarginate.....3
3. Pubescence composed of a single type of fine simple pubescent hairs .....
- 4*  
Pubescence composed of closely appressed, tomentose, or scale like deciduous hairs, and usually interspersed with more erect pubescent hairs.....8
4. Pubescence intermixed with black hairs; spinules on tibiae black .....
- 5*  
Pubescence generally unicolorous; spinules on tibiae pale.....6
5. Lateral margins of pronotum straight; prosternal xyphus immarginate; posterior femora more or less incrassate in male .....
- Plagiognathus*  
Lateral margins of pronotum more or less sinuate; prosternal xyphus tenuously marginate; posterior femora slender in male.....*Agraptocoris*
6. Rostral segment I short, not surpassing base of head; spinules at least of posterior tibiae with dark spots at base .....
- Leucodellus*  
Rostral segment I longer, attaining middle of xyphus; tibiae without dark spots .....
- 7*
7. Rostrum reaching or scarcely surpassing apex of posterior coxae, posterior tarsal segments II and III subequal in length .....
- Eucharicoris*  
Rostrum scarcely reaching apex of intermediate coxae, posterior tarsal segment II distinctly longer than III .....
- Ectenellus*
8. Head strongly produced beyond the eyes, antennal segment II incrassate (except the ♀ of *Criocoris*) .....
- 9*

- Head not or scarcely produced, inclined or subvertical in position; antennal segment II linear or nearly so.....*Psallus*
- g. Antennal segment I distinctly surpassing apex of clypeus, segment II fusiform in both sexes; lateral margins of pronotum somewhat sinuate.....*Excentricus*
- Antennal segment I not surpassing apex of clypeus, segment II strongly incrassate in the male, slender in the female; lateral margins of pronotum straight .....*Criocoris*

### GENUS CHLAMYDATUS CURTIS

#### Key to Species

1. Body densely clothed with black hairs; rostrum reaching posterior coxae .....*pachycerus*  
Body clothed with grayish pubescence; rostrum only reaching intermediate coxae .....2
  2. Antennal segments I and II chiefly black, segment II much shorter than III and IV taken together.....*pullus*  
Antennal segments I and II chiefly rusty yellow, segment II nearly as long as III and IV taken together.....*fulvicornis*
- CHLAMYDATUS PACHYCERUS** Kiritshenko  
 1931 *Chlamydatus pachycerus* Kiritshenko, Ann. Mag. Nat. Hist., (10) vii, p. 384.  
 Tibet.

**CHLAMYDATUS PULLUS** (Reuter)

1870 *Agaliastes pullus* Reuter, Not. Sällsk. F. et. Fl. Fenn. Förh., xi, 324.  
 Kansu, Mongolia.

**CHLAMYDATUS FULVICORNIS** (Jakovlef)

1890 *Agaliastes fulvicornis* Jakovlef, Hor. Soc. Ross., xxiv, p. 348.  
 Mongolia.

### GENUS STHENARUS FIEBER

#### Key to Species

1. Posterior margin of vertex distinctly carinate; tibiae with dark spots at base of spinules, apex blackish; femora with series of fuscous spots.....*pallidipes*  
Posterior margin of vertex ecarinate; tibiae unicolorous or black at base, without dark spots, femora without series of dark spots.....2
2. Head about three-fifths as wide as base of pronotum; rostrum reaching apex of posterior coxae; pronotum distinctly transversely rugulose.....*interruptus*  
Head about three-fourths as wide as base of pronotum; rostrum reaching apex of intermediate coxae; pronotum obsolete finely rugulose.....3
3. Antennal segment II strongly incrassate, apex distinctly thicker than segment I; cuneus with an arcuate white marking at base.....*niveoarcuatus*  
Antennal segment II slightly incrassate, apex as thick as segment I; cuneus concolorous .....*potanini*

**STHENARUS NIVEOARCUATUS** Reuter

1906 *Sthenarus niveoarcuatus* Reuter, Ann. Mus. Zool. St. Pet., x, p. 80.  
 Sikang.

**STHENARUS POTANINI** Reuter

1906 *Sthenarus potanini* Reuter, Ann. Mus. Zool. St. Pet., x, p. 77.  
 Szechuan.

**STHENARUS INTERRUPTUS** Reuter

1906 *Sthenarus interruptus* Reuter, Ann. Mus. Zool. St. Pet., x, p. 79.  
 Szechuan.

**STHENARUS PALLIDIPES** Reuter

1906 *Sthenarus pallidipes* Reuter, Ann. Mus. Zool. St. Pet., x, p. 78.  
 Szechuan.

## GENUS PLAGIOPGNATHUS FIEBER

## Key to Species

1. Antennal segments I and II totally black, body generally dark.....*arbustorum*  
Antennal segments I and II only partly black, body above chiefly pale.....2
2. Body above clothed with pale or flavous hairs.....3  
Body above clothed with black or fuscous hairs.....4
3. Body above densely clothed with long fragile pale hairs, rostrum not reaching apex of posterior coxae.....*albipennis*  
Body above finely flavous pubescent, rostrum reaching apex of posterior coxae .....*breviceps*
4. Body above finely pale pubescent, intermixed with fuscous hairs, rostrum barely surpassing intermediate coxae.....*lividus*  
Body above clothed only with black hairs, rostrum reaching apex of posterior coxae .....5
5. Pronotum and hemelytra usually marked with black, ventral segments black with margins greenish gray.....*cinerascens*  
Pronotum and hemelytra unicolorously pale greenish or flavous, venter fuscous or greenish.....*chrysanthemi*

## PLAGIOPGNATHUS BREVICEPS Reuter

1879 *Plagiognathus* ? *breviceps* Reuter, Hem. Gym. Eur., i, p. 82.  
Kansu, Sikang, Szechuan.

## PLAGIOPGNATHUS ALBIPENNIS (Fallén)

1829 *Phytocoris albipennis* Fallén, Hem. Suec., Cim., p. 107.  
Hopei; Liaoning\*: Mukden, Aug. 1931 (G. Liu); Szechuan.

## PLAGIOPGNATHUS ARBUSTORUM var. MOESTUS Reuter

1906 *Plagiognathus albipennis* var. *moesta* Reuter, Ann. Mus. Zool. St. Pet., x, p. 75.  
Szechuan.

## PLAGIOPGNATHUS ARBUSTORUM (Fabricius)

1794 *Lygaeus arbustorum* Fabricius, Ent. Syst., iv, p. 175.  
Szechuan.

## PLAGIOPGNATHUS ARBUSTORUM var. HORTENSIS (Meyer-Dür)

1843 *Capsus hortensis* Meyer-Dür, Verz. Schw. Rhyn. Caps., p. 66, t. 3, f. 2.  
Szechuan.

## PLAGIOPGNATHUS ARBUSTORUM var. PALLIDIPENNIS Reuter

1906 *Plagiognathus arbustorum* var. *pallidipennis* Reuter, Ann. Mus. Zool. St. Pet., x, p. 75.  
Kansu, Szechuan.

## PLAGIOPGNATHUS CINERASCENS Reuter

1904 *Plagiognathus cinerascens* Reuter, Öfv. Fin. Vet. Soc. Förh., xlvi, No. 14, p. 17.  
Mongolia.

## PLAGIOPGNATHUS LIVIDUS Reuter

1906 *Plagiognathus lividus* Reuter, Ann. Mus. Zool. St. Pet., x, p. 73.  
Szechuan.

## PLAGIOPGNATHUS CHRYSANTHEMI (Wolff)

1804 *Miris chrysanthemi* Wolff, Icones Cimicum, p. 157.  
Kansu.

## GENUS CRIOCORIS FIEBER

## CRIOCORIS QUADRIMACULATUS (Fallén)

1829 *Capsus quadrimaculatus* Fallén, Hem. Suec. Cim., p. 119.  
Mongolia.

\* New record.

## GENUS EXCENTRICUS REUTER

**EXCENTRICUS PICTIPES** Reuter1879 *Excentricus punctipes* var. *pictipes* Reuter, Hem. Gym. Eur., i, p. 91.  
Mongolia.

## GENUS PSALLUS FISHER

## Key to Species

1. Body reddish testaceous, opaque..... *opacus*  
Body black or principally so, shining..... 2
2. Antennal segment II shorter than width of pronotum at base..... *aenescens*  
Antennal segment II longer than width of pronotum..... 3
3. Antennal segment II less than three times as long as I; spinules on tibiae pale; ostiolar peritreme black and opaque..... *alpestris*  
Antennal segment II about five to six times as long as I, spinules on tibiae black; ostiolar peritreme pale..... *holomelas*

**PSALLUS OPACUS** Reuter1906 *Psallus opacus*, Reuter, Ann. Mus. Zool. St. Pet., x, p. 72.  
Sikang, Szechuan.**PSALLUS AENESCENS** (Reuter)1901 *Neocoris aenescens* Reuter, Öfv. Fin. Vet. Soc. Förh., xliii, p. 188.  
Mongolia.**PSALLUS ALPESTRIS** Reuter1906 *Psallus alpestris* Reuter, Ann. Mus. Zool. St. Pet., x, p. 70.  
Szechuan.**PSALLUS HOLOMELAS** Reuter1906 *Psallus holomelas* Reuter, Ann. Mus. Zool. St. Pet., x, p. 69.  
Szechuan.

## GENUS LEUCODELLUS REUTER

**LEUCODELLUS ALBIDUS** Reuter1906 *Leucodellus albidus* Reuter, Ann. Mus. Zool. St. Pet., x, p. 69.  
Szechuan.

## GENUS ECTENELLUS REUTER

**ECTENELLUS TIBIALIS** Reuter1906 *Ectenellus tibialis* Reuter, Ann. Mus. Zool. St. Pet., x, p. 67.  
Sikang.

## GENUS EUCHARICORIS REUTER

**EUCHARICORIS PALLIDIPENNIS** Reuter1906 *Eucharicoris pallidipennis* Reuter, Ann. Mus. Zool. St. Pet., x, p. 65.  
Szechuan.

## GENUS AGRAPTOCORIS REUTER

**AGRAPTOCORIS CONCOLOR** Reuter1904 *Agraptocoris concolor* Reuter, Öfv. Fin. Vet. Soc. Förh., xlvi, No. 4, p. 7.  
Mongolia.

## TRIBE ONCOTYLINI

## .. Key to Genera

1. Body above devoid of black hairs, pseudarolia laminate-explanate, apical margin of pronotum straight..... *Atomophora*  
Body above clothed with black hairs, pseudarolia narrow or acuminate, apical margin of pronotum more or less sinuate..... 2

2. Rostrum short, not surpassing apex of intermediate coxae, antennal segment I longly surpassing apex of head, pseudarolia narrow.....*Oncotylus*  
 Rostrum longer, reaching apex of posterior coxae, antennal segment I not surpassing apex of head, pseudarolia acuminate.....*Acrotelus*

## GENUS ATOMOPHORA Reuter

**ATOMOPHORA SUTURALIS** Reuter

1904 *Atomophora suturalis* Reuter, Öfv. Fin. Vet. Soc. Förh., xlvi, No. 4, p. 13.  
 Mongolia.

## GENUS ONCOTYLUS FIEBER

**ONCOTYLUS FUSCICORNIS** Reuter

1904 *Oncotylus fuscicornis* Reuter, Öfv. Fin. Vet. Soc. Förh., xlvi, No. 14, p. 10.  
 Mongolia.

## GENUS ACROTELUS REUTER

**ACROTELUS PILOSICORNIS** Reuter

1901 *Acrotelus pilosicornis* Reuter, Öfv. Fin. Vet. Soc. Förh., xlvi, No. 29, p. 184.  
 Mongolia.

## TRIBE ALLODAPINI

## GENUS ALLODAPUS FIEBER

**ALLODAPUS SIBIRICUS** Poppius

1912 *Allodapus sibiricus* Poppius, Öfv. Fin. Vet. Soc. Förh., liv, A, No. 29, p. 8.  
 Kansu.

## SUBFAMILY DICYPHINAE

## Key to Genera

- Transverse sulcus on pronotum behind calli extending to sides of pronotum ..... *Dicyphus* Fieb.  
 Transverse sulcus behind calli not extending to sides of pronotum...*Cyrtopeltis*

## GENUS DICYPHUS FIEBER

**DICYPHUS NIGRIFRONS** Reuter

1906 *Dicyphus nigrifrons* Reuter, Ann. Mus. Zool. St. Pet., x, p. 61.  
 Szechuan.

## GENUS CYRTOPELTIS FIEBER

## Key to Species

- Body more than 4.5 mm. long, antennal segment II as long as width of pronotum at base, scutellum and hemelytra unicolorous, vertex nearly three times as wide as eye.....*geniculata* Fieb.  
 Body less than 4 mm. long, length of antennal segment II less than width of pronotum, scutellum and hemelytra marked fuscous, vertex about as wide as eye .....*tenuis* Reut.

**CYRTOPELTIS TENUIS** Reuter

1895 *Cyrtopeltis tenuis* Reuter, Rev. d'Ent., xiv, p. 139.

1904 *Gallobelicus crassicornis* Distant, Faun. Brit. Ind., Rhyn., ii, p. 478, f. 310.  
 "China"; Shantung\*: Tsinan, Sept. 7, 1934.

\* New record.

**CYPTOPELTIS GENICULATA** Fieber

1861, *Cyrtopeltis geniculata* Fieber, Eur. Hem., p. 323.  
Kansu, Mongolia.

**SUBFAMILY BRYOCORINAE****Key to Genera**

1. Scutellum subspherical, very strongly convex; antennal segments II and III clavate, IV fusiform.....*Rhopaliceschatus*  
Scutellum and antennae otherwise constructed.....2
2. Pronotum strongly constricted at a point slightly before middle; scutellum moderately convex, sometimes with a central sulcation.....*Pachypeltis*  
Pronotum and scutellum otherwise constructed.....3
3. Eyes removed from pronotum, head constricted behind eyes...*Cobalorrhynchus*  
Eyes nearly contiguous to pronotum, head not constricted behind eyes.....4
4. Body oblong, pronotum impunctate, rostrum not reaching apex of mesosternum .....*Bryocoris*  
Body broadly ovate, pronotum finely punctate, rostrum reaching apex of mesosternum .....*Monalocoris*

**GENUS MONALOCORIS DAHLBOM****MONALOCORIS FILICIS** Linnaeus

1758 *Cimex filicis* Linnaeus, Syst. Nat., ed. x, p. 443.  
Anhwei \*; Taipinghien, Oct. 1932; Szechuan \*; Peibay, June, 1932 (G. Liu).

**GENUS BRYOCORIS FALLÉN****BRYOCORIS CONVEXICOLLIS** Hsiao

1941 *Bryocoris convexicollis* Hsiao, Iowa State College Jour. Sci., xv, No. 3, p. 241.  
Szechuan.

**GENUS COBALORRHYNCHUS REUTER****COBALORRHYNCHUS BIQUADRANGULIFER** Reuter

1906 *Cobalorrhynchus biquadrangulifer* Reuter, Ann. Mus. Zool. St. Pet., x, p. 2.  
Sikang.

**GENUS PACHYPELTIS SIGNORET****PACHYPELTIS CHINENSIS** Signoret.

1857 *Pachypeltis chinensis* Signoret, Ann. Soc. Ent. Fr., (3) vi, p. 501.  
"China."

**GENUS RHOPALICESCHATUS REUTER****RHOPALICESCHATUS QUADRIMACULATUS** Reuter

1903 *Rhopaliceschatus quadrimaculatus* Reuter, Öfv. Fin. Vet. Soc. Förh., xlv, No. 16, p. 3, t. 2, f. 1.  
Szechuan.

**SUBFAMILY DERAEOCORINAE****Key to Genera**

- Head subhorizontally produced, constricted behind eyes, eyes somewhat removed from pronotum, tarsal segment III thickened toward apex, rostrum short, slightly surpassing apex of mesosternum.....Ix
- Head not distinctly produced, tarsal segment III normal, rostrum longer, .....*Deraeocoris*

\* New record.

## GENUS IX BERGROTH

This genus was referred to the subfamily *Bryocorinae* by Bergroth in 1917. In 1926 Dr. Knight examined the type in United States National Museum and recorded the arolia as indistinct, undoubtedly bristle-like, and referred the genus to subfamily *Deraeocorinae*.

## IX PORRECTA Bergroth

1917 *Ix porrecta* Bergroth, Proc. U. S. Nat. Mus., li, p. 235.  
Hongkong.

## GENUS DERAEOCORIS KIRSCHBAUM

## Key to Species

1. Scutellum punctate, sometimes only very obsoletely so..... 2  
Scutellum impunctate..... 6
2. Collar of pronotum opaque..... 3  
Collar of pronotum shining..... 4
3. Body more than 6 mm. long, length of antennal segment I greater than width of vertex ..... *annulipes*  
Body less than 5 mm. long, length of antennal segment I less than width of vertex ..... *punctulatus*
4. Body more than 7 mm. long, length of antennal segment I greater than width of vertex ..... 5  
Body less than 5 mm. long, length of antennal segment I less than width of vertex ..... *aphidicidus*
5. Femora broadly ferruginous at apex, cuneus black or sanguinous at middle, head usually ferruginous with clypeus black..... *ruber*  
Femora entirely black, cuneus white at middle, head black with base luteus ..... *sibericus*
6. Body at least with pronotum, antennae and legs pilose..... 7  
Body above glabrous..... 8
7. Length of antennal segment I nearly twice the width of vertex, collar of pronotum slender and shining..... *montanus*  
Length of antennal segment I slightly less or greater than width of vertex; collar of pronotum wider and opaque..... *pilipes*
8. Collar of pronotum opaque; antennal segment II at least three times the length of I ..... 9  
Collar of pronotum shining; antennal segment II shorter, rarely three times the length of I, but if so then calli on pronotum strongly elevated and confluent at middle..... 10
9. Body above black, only with posterior margin of head and sometimes scutellum discolorous; rostrum reaching posterior coxae..... *scutellaris*  
Body above fuscous and flavous variegated; rostrum only reaching upon intermediate coxae ..... *horvathi*
10. Pronotum and hemelytra concolorously punctate, calli on pronotum strongly elevated ..... *aliticallus*  
Pronotum and hemelytra fusco-punctate, calli somewhat flat..... 11
11. Vertex immarginate, pronotum uniformly fulvous..... *anhwenicus*  
Vertex tenuously marginate, pronotum ochraceous and fuscous..... *nigropectus*

## DERAEOCORIS SIBERICUS Kiritschenko

1890 *Capsus ater* Jakovlef, Hor. Soc. Ent. Ross., xxiv, p. 344. (nom. preoc.).

1913 *Deraeocoris sibiricus* Kiritschenko, Rev. Russ. Ent., xiii, p. 483.  
"China".

## DERAEOCORIS RUBER (Linnaeus)

1758 *Cimex ruber* Linnaeus, Syst. Nat., ed. x, i, p. 446.

Kiangsu: Nanking, June 12, 1939.

## DERAEOCORIS SCUTELLARIS (Fabricius)

1794 *Lygaeus scutellaris* Fabricius, Ent. Syst., iv, p. 180. (nec. Reuter).  
 , Mongolia.

## DERAEOCORIS ANNULIPES (Herrich-Schäffer)

1842 *Capsus annulipes* Herrich-Schäffer, Wanz. Ins., vi, p. 97, f. 669.  
 Mongolia.

## DERAEOCORIS HORVATHI Poppius

1915 *Deraeocoris Horvathi* Poppius, Ann. Mus. Nat. Hung., xiii, p. 78.  
 Yunan.

## DERAEOCORIS APHIDICIDUS Ballard

1927 *Deraeocoris aphidicidus* Ballard, Mem. Dept. Agr. Ind. Ent. Ser., x, No. 4, p. 62,  
 pl. 16, f. 3.

This species was first reported by its author as "predacious, especially on *Aphis gossypii* Glover." The specimens on hand agree with Ballard's description and are predacious on *Thysanozyna limbata* Enderlein (kindly determined by L. D. Tuthill) according to Dr. Tsou.

Szechuan \*, 1939 (T. L. Tsou).

## DERAEOCORIS NIGROPECTUS Hsiao

1941 *Deraeocoris anhwenicus* Hsiao, Iowa State College Jour. Sci., xv, No. 3, p. 242.  
 Anhwei, Kwangsi.

## DERAEOCORIS ANHWENICUS Hsiao

1941 *Deraeocoris anhwenicus* Hsiao, Iowa State College Jour. Sci., xv, No. 3, p. 242.  
 Anhwei.

## DERAEOCORIS PUNCTULATUS (Fallén)

1807 *Lygaeus punctulatus* Fallén, Mon. Cim. Svec., p. 87.  
 Kansu.

## DERAEOCORIS PUNCTULATUS var. PULCHELLUS (Reuter)

1906 *Camptobrochis punctulatus* var. *pulchella* Reuter, Ann. Mus. Zool. St. Pet.,  
 x, p. 56.  
 Szechuan.

## DERAEOCORIS PUNCTULATUS var. POPPIUS (Reuter)

1906 *Camptobrochis punctulatus* var. *poppius* Reuter, Ann. Mus. Zool. St. Pet.,  
 x, p. 57.  
 Szechuan.

## DERAEOCORIS ALTICALLUS Hsiao

1941 *Deraeocoris alticallus* Hsiao, Iowa State College Jour. Sci., xv, No. 3, p. 243.  
 Szechuan.

## DERAEOCORIS PILIPES (Reuter)

1879 *Camptobrochis pilipes* Reuter, Öfv. Fin. Vet. Soc. Förh., xxi, p. 201.  
 Mongolia, Szechuan.

## DERAEOCORIS MONTANUS Hsiao

1941 *Deraeocoris montanus* Hsiao, Iowa State College Jour. Sci., xv, No. 3, p. 244.  
 Szechuan.

## SUBFAMILY ORTHOTYLINAE

## Key to Genera TRIBE.

1. Eyes pedunculate, head very broad..... *Labopini*  
   Eyes normal, head narrower..... 2
2. Body robust, short oval or ovate, femora saltatorial; head strongly vertical,  
   usually longly produced..... *Haltctnt*  
   Body usually elongate, femora normal, head not sharply vertical..... 3
3. Form antlike, sides of pronotum more or less sulcate-sinuate, or greatly  
   narrowed on the apical half; usually with silvery markings composed of  
   scalelike deciduous hairs..... *Pilophorint*

\* New record.

Form not antlike, sides of pronotum not sulcate-sinuate, devoid of silvery markings ..... *Orthotylini*

#### TRIBE HALTICINI

##### Key to Genera

1. Antennae very slender, longer than body, segment I not or scarcely reaching middle of clypeus, III shorter than IV; hind femora strongly incrassate..... 2  
Antennae less slender, shorter than body, segment I surpassing middle of clypeus, III longer than IV; hind femora less incrassate..... 3
2. Head broad, vertex with margin arcuate, covering apex of pronotum; eyes incumbent on anterior pronotal angles; antennal segment I not reaching middle of clypeus..... *Halticus*  
Head much narrower, vertex with margin straight, not covering apex of pronotum, eyes not incumbent on anterior pronotal angles; antennal segment I reaching middle of clypeus..... *Ectmetopterus*
3. Body clothed with simple pubescence; pronotum broadly rounded at base; length of antennal segment II not greater than width of vertex.... *Strongylocoris*  
Body clothed with scalelike deciduous hairs and interspersed with straight suberect hairs; pronotum sinuate or truncate at base; length of antennal segment II greater than width of vertex, usually much greater than width of head ..... *Orthocephalus*

#### GENUS HALTICUS HAHN

*Halticus minutus* Reuter

1884 *Halticus minutus* Reuter, Ent. Tidskr., v, p. 197.

Anhwei\*: Taipingshien, Oct. 1932 (G. Liu); Kiangsu\*: Nanking, Aug. 14, 1919 (H. F. Loomis); Szechuan: Chungtu, June 24, 1938 (K. F. Chen).

#### GENUS ECTMETOPTERUS REUTER

*Ectmetopterus angusticeps* Reuter

1906 *Ectmetopterus angusticeps* Reuter, Ann. Mus. Zool. St. Pet., x, p. 60.

Anhwei\*: Taipingshien, Oct. 1932 (G. Liu); Hopei\*: Peiping, July 1932 (G. Liu); Kiangsu\*: Nanking, 14, 1919 (H. F. Loomis); Szechuan: Chingcheng-shan, July 1932.

#### GENUS STRONGYLOCORIS BLANCHARD

*Strongylocoris leucocephalus* (Linnaeus)

1758 *Cimex leucocephalus* Linnaeus, Syst. Nat., ed. x, i, p. 446.  
Kansu.

#### GENUS ORTHOCEPHALUS FIEBER

##### Key to Species

Body length 6 mm., above with silvery squamous hairs..... *funestus*  
Body not more than 5 mm., above with ochraceous squamous hairs.\* *beresovskii*

*Orthocephalus funestus* Jakovlef

1881 *Orthocephalus funestus* Jakovlef, Bul. Soc. Nat. Mosc., lvi, (i), p. 159.  
Manchuria.

*Orthocephalus beresovskii* Reuter

1906 *Orthocephalus beresovskii* Reuter, Ann. Mus. Zool. St. Pet., x, p. 57.

1906 *Orthocephalus beresovskii* var. *fulvipes* Reuter, Ann. Mus. Zool. St. Pet., x, p. 57.  
Kansu.

\* New record.

**ORTHOCEPHALUS BERESOVSKII** var. **TIBIALIS** Reuter1906 *Orthocephalus beresovskii* var. *tibialis* Reuter, Ann. Mus. Zool. St. Pet., x, p. 58.1906 *Orthocephalus beresovskii* var. *fuscipes* Reuter, Ann. Mus. Zool. St. Pet.,  
x, p. 58.

Kansu.

**TRIBE LABOPINI****GENUS LABOPS BURMESTER****LABOPS NIGRIPES** Reuter1901 *Labops nigripes* Reuter, Öfv. Fin. Vet. Soc. Förh., xlili, p. 171.  
Mongolia.**TRIBE ORTHOTYLINI****Key to Genera**

1. Head constricted behind eyes; pronotum less than twice as wide as long, declivous anteriorly, sulcus behind calli distinct and extending over the side ..... *Cyllecoris*  
Head not constricted behind eyes, pronotum twice as wide as long, subhorizontal, sulcus behind calli less conspicuous ..... 2
2. Pubescence composed of scalelike hairs and interspersed with simple erect hairs ..... *Melanotrichus*  
Pubescence normal, composed of a single type of simple hairs ..... 3
3. Frons convex medianly, antennal segment II longer than III and IV taken together ..... *Aretas*  
Frons not convex, antennal segment II shorter than III and IV taken together .. 4
4. Head transverse, when viewed from side not or scarcely projecting below base of head, gula oblique; sexes similar ..... *Cyrtorhinus*  
Head less transverse, when viewed from side distinctly projecting below base of head, gula nearly perpendicular; sexes very dissimilar, female often brachypterous, abdomen very broad ..... *Mecomma*

**GENUS CYLLECORIS HAHN****CYLLECORIS SORDIDUS** Lindberg1934 *Cyllecoris sordidus* Lindberg, Arkiv f. Zool., 27A, No. 28, p. 40.  
Kansu.**GENUS MECOMMA FIEBER****MECOMMA CHINENSIS** Reuter1906 *Mecomma Chinensis* Reuter, Ann. Mus. Zool. St. Pet., x, p. 63.  
Szechuan.**GENUS MELANOTRICHUS REUTER****Key to Species**

- Body green, sprinkled yellowish, antennal segment III shorter than segment II ..... *flavosparsus*  
Body uniformly yellow, antennal segment III nearly as long as segment II ..... *nigropilosus*

**MELANOTRICHUS FLAVOSPARSUS** (Sahlberg)1842 *Phytocoris flavosparsus* Sahlberg, Acta Soc. Sci. Fenn., i, p. 411.

Hopei\*: Peiping, July 1932 (G. Liu); Kansu; Shantung\*: Tsinan, Aug. 1934.

\* New record.

**MELANOTRICHUS NIGROPILOSUS** (Lindberg)

1934 *Orthotylus nigropilosus* Lindberg, Arkiv f. Zool., 27A, No. 28, p. 41.  
Kansu.

#### GENUS CYRTORHINUS FIEBER

##### Key to Species

Pronotum uniformly black; antennal segment II slightly shorter than segments III and IV taken together.....*chinensis*

Pronotum with calli and a longitudinal fascia flavous; antennal segment II about two-thirds the length of segments III and IV taken together..*lividipennis*

**CYRTORHINUS CHINENSIS** (Stål)

1859 *Capsus chinensis* Stål, Freg. Eug. Resa, 1859, p. 258.  
Hongkong.

**CYRTORHINUS LIVIDIPENNIS** Reuter

1884 *Cyrtorrhinus lividipennis* Reuter, Ent. Tidskr. vi, p. 199.  
Anhwei\*: Taipingshien, Oct. 1932 (G. Liu); Chekiang\*: Hangchow, Sept. 21, 1933; Kiangsu\*: Shanghai, Sept. 21, 1936 (E. Suenson).

#### GENUS ARETAS DISTANT

**ARETAS CHINENSIS** Hsiao

1941 *Aretas chinensis* Hsiao, Iowa State College Jour. Sci., xv, No. 3, p. 245.  
Szechuan.

#### TRIBE PILOPHORINI

#### GENUS PILOPHORUS HAHN

**PILOPHORUS** sp.

Szechuan\*: Bei Bay, June 1932 (G. Liu). This species is very close to *P. javanus* Popp. (1914). The unique specimen at hand is in such poor condition that it is difficult to identify. However, it is interesting to notice that this is the first record of this cosmopolitan genus from China.

#### GENUS FULGENTIUS DISTANT

**FULGENTIUS MANDARINUS** Distant

1904 *Fulgentius mandarinus* Distant, Ann. Mag. Nat. Hist., (7), xiii, p. 104.  
Kwangtung: Namo Islands.

#### SUBFAMILY MIRINAE

##### Key to Genera

1. Scutellum distinctly punctate ..... *Stenodema*  
Scutellum impunctate ..... 2
2. Head with frons inclined, eyes slightly removed from anterior angles of pronotum, body clothed with fine, long erect hairs..... *Miris*  
Head with frons horizontal, eyes more or less in contact with apex of pronotum, body nearly glabrous ..... 3
3. Tibiae covered with long hairs and spinules indistinct; frons usually produced over base of clypeus ..... *Notoestira*  
Tibiae covered with short hairs and spinules distinct; frons not or slightly produced over base of clypeus..... *Trigonotylus*

\* New record.

## GENUS STENODEMA LAPORTE

## Key to Species

1. Posterior femora armed with spines near apex.....Subgenus *Brachytropis*  
Posterior femora without spines.....2. Subgenus *Stenodema*
2. Frons distinctly produced into a lobe over base of clypeus.....3  
Frons not produced over base of clypeus.....6
3. Posterior legs with femora strongly constricted at apex and tibiae distinctly curvate at base .....*virens*  
Posterior legs with femora not or very slightly constricted at apex and tibiae straight or nearly so .....4
4. Pronotum densely punctate, hairs on antennal segment I as long as the thickness of segment .....5  
Pronotum remotely punctate, hairs on antennal segment I shorter than the thickness of segment.....*elegans*
5. Antennal segment I three times as long as width of vertex.....*ruberinerve*  
Antennal segment I twice as long as width of vertex.....*alpestrum*
6. Posterior legs with femora strongly constricted at apex, tibiae distinctly curvate at base.....*laevigatum*  
Posterior femora not constricted at apex, posterior tibiae straight.....7
7. Body narrow, above principally greenish, antennal segment II longer than segments III and IV taken together.....*plebejum*  
Body wider, above principally fuscous, antennal segment II shorter than segments III and IV taken together.....*chinensis*

*STENODEMA VIRENS* (Linnaeus)1767 *Cimex virens* Linnaeus, Syst. Nat., ed. xii, i, p. 730.

Mongolia.

*STENODEMA VIRENS* var. *TESTACEUM* (Reuter)1875 *Miris virens* var. *testaceus* Reuter, Rev. Crit. Caps., ii, p. 3.

Mongolia.

*STENODEMA ALPESTRE* Reuter1904 *Stenodema alpestre* Reuter, Öfv. Fin. Vet. Soc. Förh., xlvi, No. 15, p. 13.

Anhwei\*: Taipingshien, Oct. 1932 (G. Liu); Szechuan: Beh Luh Din, alt. 6,000 ft., Aug. 2, 1933 (G. D. Graham); Mu Sang Tsai, alt. 8,500-10,000 ft., July 26, 1933 (D. C. Graham); Muping, alt. 4,000-7,000 ft., July 1, 1929 (D. C. Graham); Ching Cheng Shien, July 1932 (G. Liu).

*STENODEMA ELEGANS* Reuter1904 *Stenodema elegans* Reuter, Öfv. Fin. Vet. Soc. Förh., xlvi, No. 15, p. 14.

Szechuan.

*STENODEMA RUBRINERVE* Horvath1905 *Stenodema rubrinerve* Horvath, Ann. Mus. Nat. Hung., iii, p. 417.

Szechuan\*: Mt. Omei, alt. 6,000-7,500 ft., Aug. 2-7, 1921 (D. C. Graham).

*STENODEMA LAEVIGATUM* (Linnaeus)1758 *Cimex laevigatum* Linnaeus, Syst. Nat., ed. x, i, p. 449.

Kansu.

*STENODEMA PLEBEJUM* Reuter1904 *Stenodema plebejum* Reuter, Öfv. Fin. Vet. Soc. Förh., xlvi, No. 15, p. 17.

Sikang; Tibet\*: Tang Gu, alt. 13,000-14,000 ft., Aug. 3-6, 1930 (D. C. Graham).

*STENODEMA CHINENSE* Reuter1904 *Stenodema chinense* Reuter, Öfv. Fin. Vet. Soc. Förh., xlvi, No. 15, p. 19.

Szechuan.

## -GENUS NOTOSTIRA Fieber

*NOTOSTIRA POPPIUSI* Kiritshenko

Lindberg (1934) recorded this species from Tien Shan, Fu-shu-shi, and north-

\* New record.

eastern Szechuan but failed to give any citation of reference. The present writer has been unable to locate Kiritshenko's description either from *Zoological Record* or from other sources. Reuter (1911) mentioned that some specimens were sent to him with the name *N. poppiusi* by Kiritshenko, collected from Turkestan. They are similar to *N. erratica* in color pattern but differ by their pure green ground color, longer antenna and legs, and longer and erect pubescence. The hairs on the first antennal segment of the females are longer and thicker as is the case with *N. caucasica*. However, Reuter reserved his definite opinion about their specific rank and claimed it as at least a distinct subspecies of *N. caucasica*.

#### GENUS MIRIS FABRICIUS

##### MIRIS? FERRUGATUS Fallén

1807 *Miris ferrugatus* Fallén, Mon. Cim. Suec., p. 109.

A single specimen was collected by D. C. Graham from Chung-Ku, Szechuan\*, alt. 11,000 ft., July 1937. It is very similar to Fallén's species but with antennae and legs much less densely hairy and antennae also much more slender. It might be an unknown species, but the specimen was crushed so badly that no complete description could be made from it.

#### GENUS TRIGONOTYLUS FIEBER

##### TRIGONOTYLUS RUFICORNIS (Geoffroy)

1785 *Cimex ruficornis* Geoffroy, in Fourcroy, Ent. Paris., p. 209.

1902 *Megaloceraea coelestium* Kirkaldy, Trans. Ent. Soc. Lond., 1902, p. 266.

Hope\*: Peiping, July 1932 (G. Liu); Kansu; Kiangsu; Nanking; Liaoning\*: Mukden, July 1931 (G. Liu); Shangtung\*: Tsinan, Aug. 24, 1934; Szechuan.

#### SUBFAMILY CAPSINAE

##### Key to Genera

1. Hemelytra transparent, claws dentate at base.....*Isabel*  
Hemelytra normal, claws not dentate at base.....2
2. Body above and below clothed with sericeous, tomentose pubescence.....3  
Body not or only above clothed with sericeous, tomentose pubescence.....6
3. Body strongly convex, usually coarsely punctate, collar of pronotum thick.....4  
Body not convex or slightly convex, collar slender.....*Polymerus*
4. Antennal segment I usually compressed, II strongly incrassate at apex.....*Eurystylus*  
Antennal segment I normal, II linear or nearly so.....5
5. Head short, vertical; posterior tarsal segments I and II subequal in length.....*Charagochilus*  
Head strongly produced before eyes, inclined; posterior tarsal segment I distinctly shorter than II.....*Proboscidocoris*
6. Head, pronotum and scutellum clothed with very long hairs, often forming tufts on pronotum.....*Tinginotum*  
Body not clothed with long hairs.....7
7. Pronotum punctate or punctulate.....8  
Pronotum impunctate or only with fine aciculate punctures.....13
8. Vertex immarginate or only very obsoletely marginate at each side.....9  
Vertex marginate, sometimes slender at the middle.....10
9. Body above glabrous, pronotum sparsely punctulate, antennal segment II robust.....*Listonotus*  
Body above pubescent, pronotum strongly punctate, antennal segment II linear.....*Cyphodemidea*

\* New record.

10. Posterior tarsal segment I as long as II and III taken together....*Alloeotomus*  
Posterior tarsal segment I as long as or shorter than II.....11
11. Posterior tarsal segment II distinctly longer than I, as long as or almost longer  
than III .....
- Posterior tarsal segment II not or scarcely longer than I, shorter than III  
.....*Lygidea*
12. Genae high, antennal segment II incrassate at apex.....*Liocoridea*  
Genae low, antennal segment II not or rarely incrassate at apex.....*Lygus*
13. Posterior femora long, generally surpassing apex of abdomen; antennal segment  
I as long as or longer than (very rarely shorter) pronotum.....14  
Posterior femora shorter, not or rarely surpassing apex of abdomen; antennal  
segment I distinctly shorter than or rarely as long as pronotum.....15
14. Posterior femora subcylindrical, only more slender at extreme apex; antennal  
segment I devoid of rigid hairs.....*Phytocoridea*  
Posterior femora flattened, broadest before middle and tapering towards apex;  
antennal segment I provided with rigid hairs.....*Phytocoris*
15. Vertex and frons subhorizontal, gula horizontal; posterior tarsal segments I and  
II equal in length.....16  
Head vertical or inclining, gula oblique; posterior tarsal segment II longer  
than I .....
16. Frons subhorizontally produced into a lobe above the clypeus; antennal segment  
II incrassate .....
- Frons declivit anteriorly; antennal segment II cylindrical.....*Pantilius*
17. Antennal segments III and IV distinctly thinner than II.....18  
Antennal segments III and IV not or very little thinner than II.....19
18. Head vertical, distinctly transverse as seen from above, scutellum exposed at  
base .....
- Head inclining, rarely slightly shorter than wide; scutellum at least narrowly  
covered at base .....
19. Lateral margins of pronotum distinctly acute, body above nearly glabrous  
.....*Philostephanus*  
Lateral margins of pronotum rounded, body above pubescent.....20
20. Clypeus seen from the side very prominent, antennal segment I distinctly  
longer than head.....*Creontiades*  
Clypeus seen from the side not prominent, antennal segment I usually as long  
as or shorter than head.....21
21. Pronotum and scutellum strongly convex, calli not distinguished, collar with  
erect rigid setae .....
- Pronotum and scutellum not or slightly convex, calli distinct, collar without  
erect rigid setae .....

#### GENUS ISABEL Kirk.

ISABEL RAVANA (Kirby)

1891 *Capsus ravana* Kirby, J. Linn. Soc. Zool., xxiv, p. 106.

Anhwei\*: Taipingshien, Oct. 1932 (G. Liu).

#### GENUS PANTILIUS CURTIS

PANTILIUS GONOCEROIDES Reuter

1903 *Pantilius gonoceroides* Reuter, Öfv. Fin. Vet. Soc. Förh., xlv, No. 16, p. 4.  
Szechuan.

#### GENUS PARAPANTILIUS REUTER

PANTILIUS GONOCEROIDES Reuter

1903 *Parapantilius thibetanus* Reuter, Öfv. Fin. Vet. Soc. Förh., xlv, No. 16, p. 6,  
t. 2, f. 2.  
Szechuan.

\* New record.

## GENUS ALLOEOTOMUS FIEBER

**ALLOEOTOMUS CHINENSIS** Reuter

1903 *Alloeotomus chinensis* Reuter, Öfv. Fin. Vet. Soc. Förh., xlv, No. 6, p. 20.  
Hopei.

## GENUS LIISTONOTUS REUTER

**LIISTONOTUS XANTHOMELAS** Reuter

1906 *Liistonotus xanthomelas* Reuter, Ann. Mus. Zool. St. Pet., x, p. 55.  
Kansu.

## GENUS LIOCORIDEA REUTER

## Key to Species

- Antennal segment II shorter than width of pronotum at base, strongly incrassate apically; spinules on tibiae black.....*mutabilis*  
 Antennal segment II about as long as width of pronotum at base, linear; spinules on tibiae pale.....*Melanostoma*

**LIOCORIDEA MUTABILIS** Reuter

1903 *Licocoridea mutabilis* Reuter, Öfv. Fin. Vet. Soc. Förh., xlv, No. 16, p. 14, t. 2,  
f. 4.  
Szechuan.

**LIOCORIDEA MUTABILIS** var. **NIGRA** Reuter

1903 *Licocoridea mutabilis* var. *nigra* Reuter, Öfv. Fin. Vet. Soc. Förh., xlv, No. 16,  
p. 15.  
Szechuan.

**LIOCORIDEA MUTABILIS** var. **TESTACEA** Reuter

1903 *Licocoridea mutabilis* var. *testacea* Reuter, Öfv. Fin. Vet. Soc. Förh., xlv, No. 16,  
p. 15.  
Szechuan.

**LIOCORIDEA MELANOSTOMA** Reuter

1906 *Licocoridea melanostoma* Reuter, Ann. Mus. Zool. St. Pet., x, p. 53.  
Szechuan.

## GENUS CYPHODEMIDEA REUTER

**CYPHODEMIDEA VARIEGATA** Reuter

1903 *Cyphodemidea variegata* Reuter, Öfv. Fin. Vet. Soc. Förh., xlv, No. 16, p. 19, t. 2,  
f. 5.  
Szechuan.

## GENUS LYGIDEA REUTER

**LYGIDEA ILLOTA** (Stal)

1858 *Deraeocoris illotus* Stal, Stett. Ent. Zeit., 1858, p. 184.  
Mongolia.

## GENUS PHILOSTEPHANUS DISTANT

**PHILOSTEPHANUS VITALITER** Distant

1909 *Philostephanus vitaliter* Distant, Ann. Mag. Nat. Hist., (8), vi, p. 449.  
Szechuan\*: Mt. Omei, Sept. 21, 1938 (K. F. Chen); Aug. 19, 1934 (D. C. Graham).

\* New record

## GENUS ADELPHOCORIS REUTER

## Key to Species

1. Body above clothed with black hairs.....2  
Body above without black hairs.....7
2. Body above uniformly bluish green, beneath uniformly greenish.....*glaucus*  
Body above otherwise colored, with more or less dark markings, beneath dark or pale .....3
3. Body beneath chiefly black, opaque, only with margin of acetabulum and ostiolar peritreme white; scutellum black, with apex testaceous and a median line fuscous .....*torquatus*  
Body beneath not black, scutellum nearly unicolorous.....4
4. Antennal segment II in length equal to width of pronotum at base, segment I much shorter than head.....*divergens*  
Antennal segment II in length distinctly greater than width of pronotum at base, segment I about as long as head.....5
5. Head more than half as wide as pronotum at base, rostrum reaching apex of intermediate coxae.....*fasciiger*  
Head less than half as wide as pronotum at base, rostrum reaching apex of posterior coxae, rarely only reaching apex of intermediate coxae ( $\delta$  of *quadripunctatus*), in this case pronotum with four black spots.....6
6. Dorsal surface of abdomen pale, fuscous-fasciated; body beneath unicolorously flavous, veins on membrane yellowish.....*quadripunctatus*  
Dorsal surface of abdomen black, venter usually blackish at middle, veins on membrane blackish .....*annulicornis*
7. Head black .....8  
Head yellowish or ferruginous, rarely with clypeus black.....13
8. Pronotum chiefly pale yellow, with black spots or band posteriorly.....9  
Pronotum blackish or only with posterior margin pale.....10
9. Body 9-11 mm. long, scutellum black with apex yellow, legs reddish brown, femora darker than tibiae.....*transversus*  
Body less than 9 mm. long, scutellum yellowish with basal margin black, legs grayish yellow, femora conspurcated ferruginous.....*melanocephalus*
10. Body above clothed with silvery pubescence; scutellum with apex white .....*apicalis*  
Body above clothed with golden pubescence; scutellum unicolorous.....11
11. Antennal segment I shorter than head, II about three times as long as I .....*seticornis*  
Antennal segment I about as long as the head, II less than three times as long as I .....12
12. Antennal segment II as long as width of pronotum at base, segment III about three-fourths as long as II .....*triannulatus*  
Antennal segment II longer than width of pronotum at base, segment III about five-sixths as long as II .....*funestus*
13. Antennal segment I much shorter than head .....*luridus*  
Antennal segment I nearly as long as or a little longer than head.....14
14. Head ferruginous, clypeus black; antennae unicolorously ferruginous .....*ticinensis* var. *suturalis*  
Head unicolorously pale yellow, antennae pallid, ferruginous or fuscous towards apex .....15
15. Antennal segment III as long as width of pronotum at base .....*taeniphorus*  
a. Pronotum with basal band, hemelytra with a cuneus-shaped area behind middle of corium, and apex of cuneus black.....typical *taeniphorus*  
b. Pronotum with basal band distinct, scutellum and hemelytra pallid, innotate, only apex of cuneus black.....var. *impictipennis*  
c. Pronotum without basal band, hemelytra marked as in typical species .....var. *defectus* Reut.

- d. Above entirely pallid, innotate, only apex of cuneus black.....var. *pallidus*  
 Antennal segment III shorter than width of pronotum at base.....16
16. Hemelytra with lateral margins concolorous; antennal segments III and IV ferruginous or fuscous, distinctly pale flavous at base.....*fasciaticollis*  
 Hemelytra with lateral margins distinctly black; antennal segments III and IV unicolorous .....*lineolatus*
- a. Above unicolorous or only with cubital vein or corium infuscated apically .....
- b. Scutellum with two longitudinal marks on middle blackish.....typical *lineolatus*
- c. Scutellum marked as the typical species, pronotum with two round spots posteriorly blackish .....
- d. Like var. *binotatus*, but calli and sometimes posterior angles of pronotum blackish .....
- .....var. *bispunctatus*

**ADELPHOCORIS SETICORNIS** (Fabricius)1775 *Cimex seticornis* Fabricius, Syst. Ent., p. 725.

Anhwei\*: Taipingshien, Oct. 1932; Kiuhua Shan, Sept. 1932 (G. Liu); Szechuan: Kuanshien, 2,000 ft., Nov. 20, 1933; Tseo Jia Geo, Sept. 2, 3, 1929; Weichow, 5,500-7,000 ft., 1923 (D. C. Graham); Chengtu, May and Sept. 27, 1938 (K. F. Chen).

**ADELPHOCORIS TORQUATUS** Reuter

1906 *Adelphocoris torquatus* Reuter, Ann. Mus. Zool. St. Pet., x, p. 12.  
 Sikang: Ta-tsien-lu; Szechuan\*: Mt. Omei, Sept. 19, 1938 (C. S. Tsai).

**ADELPHOCORIS APICALIS** Reuter

1906 *Adelphocoris apicalis* Reuter, Ann. Mus. Zool. St. Pet., x, p. 13.  
 Szechuan.

**ADELPHOCORIS FUNESTUS** Reuter

1903 *Adelphocoris funestus* Reuter, Öfv. Fin. Vet. Soc. Förh., xlv, No. 16, p. 7.  
 Szechuan: Mou-Pin, 4,000-7,000 ft., Aug. 25, 1929; Yachow to Muping, June 23, 1929, 2,000-5,000 ft.; Behluh Din Sept. 20-28, 1934, 6,000 ft. (D. C. Graham).

**ADELPHOCORIS FUNESTUS** var. **RUFIPES** Reuter

1906 *Adelphocoris funestus* var. *rufipes* Reuter, Ann. Mus. Zool. St. Pet., x, p. 11.  
 Szechuan.

**ADELPHOCORIS TRIANNULATUS** (Stål)

1858 *Deraeocoris triannulatus* Stål, Stett. Ent. Zeit., xix, p. 183.  
 Kansu.

**ADELPHOCORIS DIVERGENS** Reuter

1906 *Adelphocoris divergens* Reuter, Ann. Mus. Zool. St. Pet., x, p. 13.  
 Szechuan.

**ADELPHOCORIS LURIDUS** Reuter

1906 *Adelphocoris luridus* Reuter, Ann. Mus. Zool. St. Pet., x, p. 14.  
 Szechuan.

**ADELPHOCORIS LURIDUS** var. **CINCTICORNIS** Reuter

1906 *Adelphocoris luridus* var. *cincticornis* Reuter, Ann. Mus. Zool. St. Pet., x, p. 15.  
 Hopei, Szechuan.

**ADELPHOCORIS TICINENSIS** var. **SUTURALIS** (Jakovlef)

1882 *Calocoris suturalis* Jakovlef, Horae Soc. Ent. Ross., xiii, p. 169.  
 Szechuan.

**ADELPHOCORIS FASCIIGER** Reuter

1906 *Adelphocoris fasciiger* Reuter, Ann. Mus. Zool. St. Pet., x, p. 17.  
 Szechuan: Weichow, 5,500 ft., 1933.

**ADELPHOCORIS TAENIOPHORUS** Reuter

1906 *Adelphocoris taeniophorus* Reuter, Ann. Mus. Zool. St. Pet., x, p. 18.  
 Szechuan.

\* New record

**ADELPHOCORIS TAENIOPHORUS var. IMPICZIPENNIS Reuter**

1906 *Adelphocoris taeniophorus* var. *impiczipennis* Reuter, Ann. Mus. Zool. St. Pet., x, p. 18.  
Szechuan.

**ADELPHOCORIS TAENIOPHORUS var. DEFECTUS Reuter**

1906 *Adelphocoris taeniophorus* var. *defectus* Reuter, Ann. Mus. Zool. St. Pet., x, p. 19.  
Szechuan.

**ADELPHOCORIS TAENIOPHORUS var. PALLIDUS Reuter**

1906 *Adelphocoris taeniophorus* var. *pallida* Reuter, Ann. Mus. Zool. St. Pet., x, p. 19.  
Szechuan.

**ADELPHOCORIS LINEOLATUS (Goeze)**

1778 *Cimex lineolatus* Goeze, Ent. Beytr., ii, p. 287.

Anhwei\*: Kiuhua Shan, April 1932 (G. Liu); Hupei\*: Hanchow, Sept. 17, 1923;  
Kansu; Kiangsu\*: Shanghai, Aug.-Oct. 1936 (E. Suenson); Mongolia; Shang-tung\*: Tsinan, July 23, 1934; Szechuan: Bu Lan Tsen, alt. 8,500-9,000 ft.,  
July 31-Aug. 2, 1933, Yachow, Aug. 1928, Weichow, alt. 5,800 ft., Aug. 1933,  
Shinkaisi, Mt. Omei, alt. 4,000-6,000 ft., Aug. 7, 1929, Kuanshien, Aug. 1-2,  
1934, Kiating, alt. 1,300 ft., Aug. 12, 1929, Wen Chuan-Shien, alt. 5,000-7,500  
ft., Aug. 24, 1933, Muping, 4,000-7,000 ft., July 1929, O-Er, 6,000 ft., Aug.  
26, 1933, (D. C. Graham), Chengtu, Aug. 2, 1938 (K. F. Chen), Ching-chengshien, July 1932 (G. Liu).

**ADELPHOCORIS LINEOLATUS var. IMPLAGIATUS (Westhoff)**

1880 *Calocoris chenopodii* var. *implagiata* Westhoff, 9 Jahrest. Schles. Vers. Wiss.  
u. Kunst., p. 74.  
Szechuan.

**ADELPHOCORIS LINEOLATUS var. BINOTATUS (Hahn)**

1833 *Phytocoris binotatus* Hahn, Wanz. Ins., i, p. 202, f. 103.  
Szechuan.

**ADELPHOCORIS LINEOLATUS var. BISBIPUNCTATUS (Reuter)**

1891 *Calocoris lineolatus* var. *bisbipunctatus* Reuter, Öfv. Fin. Vet. Soc. Förh.,  
xxxii, p. 189.  
Mongolia; Szechuan.

**ADELPHOCORIS ANNULICORNIS (Sahlberg)**

1848 *Capsus annulicornis* Sahlberg, Mon. Geoc. Fenn., p. 100.  
Mongolia.

**ADELPHOCORIS ANNULICORNIS var. CONFLUENS Reuter**

1896 *Adelphocoris annulicornis* var. *confluens* Reuter, Hem. Gym. Eur., v, p. 226.  
Mongolia.

**ADELPHOCORIS QUADRIPIUNCTATUS (Fabricius)**

1794 *Lygeus quadripunctatus* Fabricius, Ent. Syst., iv, p. 172.  
Anhwei\*: Taipingshien, Oct. 1932 (G. Liu); Szechuan: Mu-Sang-Tsai, alt.  
8,900-10,000 ft., July 26, 1933, Weichow, alt. 5,500 ft., 1933 (D. C. Graham).

**ADELPHOCORIS QUADRIPIUNCTATUS var. INNOTATUS Reuter**

1906 *Adelphocoris quadripunctatus* var. *innotata* Reuter, Ann. Mus. Zool. St. Pet.,  
x, p. 20.  
Szechuan.

**ADELPHOCORIS QUADRIPIUNCTATUS var. SCUTELLARIS Reuter**

1906 *Adelphocoris quadripunctatus* var. *scutellaris* Reuter, Ann. Mus. Zool. St. Pet.,  
x, p. 21.  
Szechuan.

**ADELPHOCORIS FASCIATICOLLIS Reuter**

1903 *Adelphocoris fasciaticollis* Reuter, Öfv. Fin. Vet. Soc. Förh., xlvi, No. 16, p. 8.  
Hopei: Peiping, July 1932 (G. Liu); Kiangsi; Shangtung\*: Tsinan, July 23,  
1934; Szechuan\*: Chengtu, Oct. 1924.

**ADELPHOCORIS GLAUCUS Hsiao**

1941 *Adelphocoris glaucus* Hsiao, Iowa State College Jour. Sci., xv, No. 3, p. 248.  
Szechuan.

**ADELPHOCORIS MELANOCEPHALUS Reuter**

1903 *Adelphocoris melanocephalus* Reuter, Öfv. Fin. Vet. Soc. Förh., xlv, No. 16, p. 9.  
Hopei.

**ADELPHOCORIS TRANSVERSUS Lindberg**

1934 *Adelphocoris transversus* Lindberg, Arkiv f. Zool., 27A, No. 28, p. 35, t. 4, f. 3.  
Kansu.

**GENUS CREONTIADES Distant****CREONTIADES PICEUS (Reuter)**

1906 *Pantiliodes piceus* Reuter, Ann. Mus. Zool. St. Pet., x, p. 7.  
Szechuan.

**CREONTIADES PALLIDIFER (Walker)**

1873 *Capsus stramineus* Walker, Cat. Hem. Het., vi, p. 120.

1873 *Capsus pallidifer* Walker, Cat. Hem. Het., vi, p. 199.  
Yunan.

**GENUS CALOCORIS FIEBER****CALOCORIS MARMORATUS Lindberg**

1934 *Calocoris marmoratus* Lindberg, Arkiv f. Zool., 27 A, No. 28, p. 36, t. 4, f. 4.  
Kansu.

**GENUS MERMITELOCERUS REUTER****MERMITELOCERUS ANNULIPES Reuter**

1908 *Mermitelocerus annulipes* Reuter, Ann. Mus. Zool. St. Pet., xii, p. 490.  
Manchuria.

**GENUS TRICHOPHORONCUS REUTER****TRICHOPHORONCUS ALBONOTATUS (Jakovlef)**

1881 *Calocoris albonotatus* Jakovlef, Bul. S. N. Mosc., i, p. 194.  
Anhwei\*: Taipingshien, Oct. 1932 (G. Liu); Kiangsi; Szechuan.

**GENUS LYGUS HAHN****Key to Species**

1. Vertex posteriorly immarginate or only laterally obsoletely marginate, spinules on tibiae pale..... 2  
Vertex posteriorly carinate or slenderly marginate, rarely immarginate but in such case the spinules on tibiae dark..... 4
2. Body more than 5.5 mm. long, scutellum concolorous with pronotum..... 3  
Body small, length 3 mm., scutellum black..... *minutus*
3. Antennal segment I and base of II without fuscous, rostrum reaching apex of posterior coxae, femora without fuscous rings..... *pabulinus*  
a. Pronotum distinctly rugose-punctate, tibiae concolorous, only tarsi at apex or entirely fuscous..... typical *pabulinus*  
b. Pronotum finely and obsoletely punctulate, tibiae at apex and tarsi entirely fuscous ..... var. *chloris*
- Antennal segment I on underside and II at base black, posterior femora subapically fuscous or ferruginous, rostrum not surpassing apex of intermediate coxae ..... *striicornis*  
a. Scutellum and legs concolorous with body, posterior femora with two subapical rings fuscous or fusco-ferruginous..... typical *striicornis*

\* New record

- b. Scutellum fusco-ferruginous, femora quite widely reddish ferruginous at apex, rings scarcely distinct.....var. *fuscoscutellatus*
- 4. Antennal segment II in length not greater than three-fourths the width of pronotum at base.....16  
Antennal segment II in length greater than three-fourths the width of pronotum .....5
- 5. Rostrum reaching apex of posterior coxae, rarely shorter, but in this case antennal segment II two and one-half times as long as I.....6  
Rostrum only reaching apex of intermediate coxae, antennal segment II about three times as long as I.....11
- 6. Body large, length more than 7.5 mm., antennal segment II at least one-sixth longer than width of pronotum at base.....7  
Body smaller, length less than 5 mm., antennal segment II not or slightly longer than width of pronotum at base.....8
- 7. Vertex as wide as eye, pronotum and scutellum opaque, antennal segment II about three times as long as I.....*rugosicollis*  
Vertex wider than eye, pronotum and scutellum shining, antennal segment II less than three times as long as I.....*longipennis*
- 8. Body principally black or with head and anterior part of pronotum reddish  
.....*szechuanensis*
  - a. Body uniformly dark.....typical *szechuanensis*
  - b. Head, pronotum anteriorly, antennae, rostrum and anterior pair of legs reddish .....

Body chiefly testaceous or flavous.....9
- 9. Spinules on tibiae robust, black, with large dark spots at base; antennal segment II as long as or slightly shorter than III and IV taken together.....*adustus*
  - a. Body less than 5 mm. long, exterior margin of corium concolorous or very slenderly black.....var. *pulchellus*
  - b. Body more than 5 mm. long, exterior margin of corium widely black .....

Spinules on tibiae slender, without dark spots at base; antennal segment II longer than III and IV taken together.....10
- 10. Vertex at each side near eyes and with posterior margin, pronotum with calli and posterior angles black, embolar margins unicolorously pale....*lindbergi*  
Vertex and pronotum innotate, embolar margins narrowly black or fuscous .....
- 11. Body chiefly fuscous or blackish.....15  
Body greenish or yellowish.....12
- 12. Body length 7.2 mm., clothed with long pubescence; head, scutellum and hemelytra marked sanguineous.....*potanini* Reuter  
Body length less than 6.5 mm., clothed with short pubescence; head, scutellum and hemelytra without sanguineous markings.....13
- 13. Spinules on tibiae with black spots at base.....*nigronasutus* Stal  
Spinules on tibiae without black spots at base.....14
- 14. Apex of cuneus black.....*spinolae*  
Apex of cuneus concolorous.....*lucorum*
- 15. Hemelytra densely clothed with long golden pubescence; spinules on tibiae black, with dark spots at base.....*dasypterus*  
Hemelytra clothed with grayish or silvery pubescence, spinules on tibiae without dark spots at base .....
- a. Width of head equal to half of width of pronotum at base; body 5 mm. long .....
- b. Width of head equal to two-fifths of width of pronotum at base, pronotum more dilatate, body 7 mm. long.....var. *duplicatus*
- 16. Antennal segment II strongly incrassate, rostrum reaching apex of posterior coxae .....

- Antennal segment II not or slightly incrassate, rostrum not reaching apex of posterior coxae or rarely so.....18
17. Antennal segment II strongly incrassate at apical half, apical two-fifths black; spinules on tibiae fuscous.....*clavicornis*  
Antennal segment II strongly incrassate with whole length, black, with a pale ring at basal fourth; spinules on tibiae testaceous .....*validicornis*
18. Embolar margin concolorous with hemelytra, never black.....19  
Embolar margin narrowly black.....20
19. Antennal segment II longer than width of head, scutellum with three longitudinal lines black.....*trivittulatus*  
Antennal segment II as long as or shorter than width of head, scutellum with an obsolete median longitudinal pale line.....*rubicundus*
20. Spinules on anterior tibiae barely distinguished, those on intermediate and posterior tibiae pale, rarely dark, in such case rostrum not reaching apex of intermediate coxae.....21  
Spinules on tibiae distinct and dark, rostrum reaching apex of intermediate coxae in female or reaching apex of posterior coxae in male.....*pratensis*
21. Spinules on tibiae dark, antennal segment II at last one-fifth longer than width of head.....*kalmiti*  
Spinules on tibiae pale, antennal segment II not or slightly longer than width of head.....22
22. Body above pale grayish ochraceous, pubescence pale, antennal segment II as long as half the width of pronotum at base.....*mutans*  
Body above black, pubescence yellowish, antennal segment II more than half as long as width of pronotum at base.....*bianchii*

**LYGUS PABULINUS (Linnaeus)**

1761 *Cimex pabulinus* Linnaeus, Fauna Suec., ed. 2, p. 253.  
Szechuan.

**LYGUS STRIICORNIS Reuter**

1906 *Lygodia striicornis* Reuter, Ann. Mus. Zool. St. Pet., x, p. 31.  
Szechuan.

**LYGUS STRIICORNIS var. FUSCOSCUTELLATUS Reuter**

1906 *Lygodia striicornis* var. *fuscoscutellatus* Reuter, Ann. Mus. Zool. St. Pet., x, p. 32.  
Sikang; Szechuan.

**LYGUS MINUTUS Hsiao**

1941 *Lygus minutus* Hsiao, Iowa State College Jour. Sci., xv, No. 3, p. 245.  
Szechuan.

**LYGUS RUGOSICOLLIS Reuter**

1906 *Lygus rugosicollis* Reuter, Ann. Mus. Zool. St. Pet., x, p. 28.  
Szechuan.

**LYGUS LONGIPENNIS Reuter**

1906 *Lygus longipennis* Reuter, Ann. Mus. Zool. St. Pet., x, p. 29.  
Sikang, Szechuan.

**LYGUS LUCORUM (Meyer-Dür)**

1843 *Capsus lucorum* Meyer-Dür, Verz. Schw. Rhyn., Caps., p. 46, pl. 6, f. 2.  
Chekiang\*: Hangchow, Sept. 11, 1933; Hopei\*: Peiping, (G. Liu); Kwangsi\*: Lungchow, April 1933, Pingloo, May 1933 (G. Liu); Szechuan: Suifu, Dec. 1924, Wen Chuan Shien, alt. 7,700 ft., Aug. 26, 1933 (D. C. Graham); Chengtu, Oct. 13, 1928 (K. F. Chen).

**LYGUS SPINOLAE (Meyer-Dür)**

1841 *Capsus spinolae* Meyer-Dür, Stett. Ent. Zeit., ii, p. 86.  
Kansu, Szechuan.

**LYGUS NIGRONASUTUS (Stål)**

1858 *Deraeocoris nigro-nasutus* Stål, Stett. Ent. Zeit., xix, p. 184.  
Kansu, Szechuan.

- LYGUS ADUSTUS** var. **PULCHELLUS** Reuter  
 1906 *Lygus pulchellus* Reuter, Ann. Mus. Zool. St. Pet., x, p. 33.  
 Szechuan.
- LYGUS ADUSTUS** var. **NIGROCINCTUS** Reuter  
 1906 *Lygus pulchellus* var. *nigrocincta* Reuter, Ann. Mus. Zool. St. Pet., x, p. 34.  
 Szechuan.
- LYGUS POTANINI** Reuter  
 1906 *Lygus potanini* Reuter, Ann. Mus. Zool. St. Pet., x, p. 35.  
 Szechuan.
- LYGUS DASYPTERUS** Reuter  
 1906 *Lygus dasypterus* Reuter, Ann. Mus. Zool. St. Pet., x, p. 37.  
 Sikang.
- LYGUS TRIVITTULATUS** Reuter  
 1906 *Lygus trivittulatus* Reuter, Ann. Mus. Zool. St. Pet., x, p. 39.  
 Sikang, Szechuan.
- LYGUS PRATENSIS** (Linnaeus)  
 1758 *Cimex pratensis* Linnaeus, Syst. Nat., ed. x, p. 448.  
 Honan\*: Kaifeng, April 1932 (G. Liu); Kansu; Mongolia; Sikang; Sinkiang;  
 Szechuan: Mou-Ping 12,000-14,000 ft., July 7, 1927 (D. C. Graham);  
 Tibet\*: Zya-Ha Pass, 14,000-17,000 ft., July 25, 27, 1930 (D. C. Graham).
- LYGUS PRATENSIS** var. **GEMELLATUS** (Herrick-Schaeffer)  
 1835 *Capsus gemellatus* Herrick-Schaeffer, Nom. Ent., i, p. 51.  
 Sikang, Szechuan: Wei Chow, 5,800 ft., Aug. 1930-; near Mouping, 1,300-1,400  
 ft., July 7, 1929 (D. C. Graham).
- LYGUS PRATENSIS** var. **PUBESCENS** Reuter  
 1807 *Lygaeus compestris* Fallen, Mon. Cim. Sue., p. 83.  
 1912 *Lygus pratensis* var. *pubescens* Reuter, Öfv. Fin. Vet. Soc. Förh., liv, Afd, A,  
 No. 7, p. 37.  
 Sikang; Szechuan.
- LYGUS PRATENSIS** var. **DISCREPANS** Reuter  
 1906 *Lygus pratensis* var. *discrepans* Reuter, Ann. Mus. Zool. St. Pet., x, p. 39.  
 Sikang, Szechuan.
- LYGUS PRATENSIS** var. **PUNCTATUS** (Zetterstedt)  
 1840 *Phytocoris punctatus* Zetterstedt, Ins. Lapp. Column 273.  
 Szechuan: O-Er, 9,000 ft., 1933, Bu Lan Tsen, 8,500-9,000 ft., July 31-Aug. 2,  
 1933 (D. C. Graham).
- LYGUS SZECHUANENSIS** Hsiao  
 1941 *Lygus szechuanensis* Hsiao, Iowa State College Jour. Sci., xv, No. 3, p. 246.  
 Szechuan.
- LYGUS CLAVICORNIS** Reuter  
 1906 *Lygus clavicornis* Reuter, Ann. Mus. Zool. St. Pet., x, p. 41.  
 Szechuan.
- LYGUS VALDICORNIS** Reuter  
 1906 *Lygus validicornis* Reuter, Ann. Mus. Zool. St. Pet., x, p. 42.  
 Sikang, Szechuan.
- LYGUS DISTINGUENDUS** Reuter  
 1875 *Lygus distinguendus* Reuter, Pet. Nouv. Ent., i, p. 544.  
 Szechuan.
- LYGUS DISTINGUENDUS** var. **DUPPLICATUS** Reuter  
 1906 *Lygus distinguendus* var. *duplicatus* Reuter, Ann. Mus. Zool. St. Pet., x, p. 47.  
 Sikang, Szechuan.
- LYGUS RUMICUNDUS** (Fallén)  
 1829 *Phytocoris rubicundus* Fallen, Hem. Suec. Cimic., p. 92.  
 Mongolia.

**LYGUS LINDBERGI n. n.**

A new name to replace *Lygus scutellatus* Lindberg (nec Distant) which is preoccupied by *Orthops scutellatus* Uhler (Bul. U. S. Sur., iii, p. 420, 1877), a synonym of *Lygus campestris* Linn.

1934 *Lygus scutellatus* Lindberg, Arkiv f. Zool. 27 A, No. 28, p. 37, t. 3, f. 5.  
Kansu.

**LYGUS CERVINUS** (Herrich-Schaeffer)

1842 *Capsus cervinus* Herrich-Schaeffer, Wanz. Ins., vi, p. 57, f. 617.  
Szechuan.

**LYGUS MUTANS** (Stal)

1858 *Deraeocoris mutans* Stal, Stett. Ent. Zeit., xix, p. 186.  
Kansu; Mongolia; Tibet\*: Tang Gu, 13,500 ft., Aug. 19, 1930 (D. C. Graham).

**LYGUS BIANCHII** Reuter

1906 *Lygus bianchii* Reuter, Ann. Mus. Zool. St. Pet., x, p. 44.  
Szechuan.

**LYGUS KALMII** (Linnaeus)

1758 *Cimex Kalmii* Linnaeus, Syst. Nat., ed. x, p. 448.  
Kansu.\*

**LYGUS KALMII** var. **THORACICUS** Westhoff

1880 *Lygus kalmi* var. *thoracicus* Westhoff, 9 Jahr. Westf. Prov. Ver. Wiss. u. Kunst., p. 68.

Sikang.

**LYGUS KALMII** var. **FERRUGINEUS** Reuter

1906 *Lygus kalmi* var. *ferruginea* Reuter, Ann. Mus. Zool. St. Pet., x, p. 46.  
Kansu, Sikang.

**LYGUS KALMII** var. **VITTICEPS** Reuter

1906 *Lygus kalmi* var. *vitticeps* Reuter, Ann. Mus. Zool. St. Pet., x, p. 46.  
Sikang, Szechuan.

## GENUS POLYMERUS HAHN

## Key to Species

1. Body above opaque, clothed with silvery tomentose pubescence, pubescence rarely yellowish, in this case rostrum surpassing apex of intermediate coxae ..... 2
- Body above shining clothed with golden tomentose pubescence ..... 3
2. Length of antennal segment II greater than width of pronotum at base, less than twice the length of segment III; base of cuneus pale yellowish... *cognatus*  
Length of antennal segment II less than width of pronotum at base, greater than twice the length of segment III; cuneus totally black ..... *pekinensis*
3. Scutellum with base black and apex yellowish; rostrum testaceous with apex black ..... *unifasciatus*  
Scutellum and rostrum entirely black ..... *funestus*

**POLYMERUS PEKINENSIS** Horvath

1900 *Polymerus pekinensis* Horvath, Zool. Erg. dritt. asiat. Forschungsreise d. Graf. Zichy, II, p. 267 (?).  
Anhwei\*: Taipingshien, Oct. 1932 (G. Liu); Hopei: Peiping; Shensi\*, July 4, 1936 (E. Suenson); Szechuan.

\* New record

\* This species together with its varieties *flavovarius* (Fabr.) and *pauperatus* (Her.-Sch.) has been recorded by Dr. C. F. Wu (Cat. Ins. Sin., ii, p. 508-509) as occurring in Manchuria, but so far as the present writer is aware there is no reference concerning this locality. Furthermore, Oshanin (Ver. Pal. Hem., i, p. 729) gave its distribution as "Tota regio palaeartica usque ad 63° (in subregione mandshurica, Japonia septentrionali excepta, tamen non inventa); . . ." However, since it is a widely distributed species it is very likely to be found in Manchuria which lies between Japan and Siberia.

**POLYMERUS FUNESTUS** (Reuter)

1908 *Poeciloscytus funestus* Reuter, Ann. Mus. Zool. St. Pet., x, p. 48.  
 ' Sikang, Szechuan.

**POLYMERUS COGNATUS** (Fieber)

1858 *Poeciloscytus cognatus* Fieber, Wien. Ent. Mon. ii, p. 331.  
 Szechuan.

**POLYMERUS UNIFASCIATUS** (Fabricius)

1894 *Lygaeus unifasciatus* Fabricius, Ent. Syst., iv, p. 178.  
 Mongolia.

**GENUS CHARAGOCHILUS** FIEBER**Key to Species**

Body more than 5 mm. long, somewhat shining, calli of pronotum strongly elevate, antennal segment II two and one-half times as long as I....*duplicatus*

Body less than 4 mm. long, opaque, calli of pronotum moderately elevate, antennal segment II three times as long as I.....*gyllenhalii*

**CHARAGOCHILUS GYLLENHALII** (Fallen)

1807 *Lygaeus gyllenhalii* Fallen, Mon. Cim. Svec., p. 88.  
 Kiangsi, Sikang, Szechuan.

**CHARAGOCHILUS DUPLICATUS** Reuter

1903 *Charagochilus duplicatus* Reuter, Öfv. Fin. Vet. Soc. Förh., xlv, No. 16, p. 16.  
 Hopei.

**GENUS PROBOSCIDOCORIS** REUTER**PROBOSCIDOCORIS LONGICORNIS** (Reuter)

1884 *Charagochilus longicornis* Reuter, Ent. Tidsk., v, p. 196.

Anwei\*: Kiuhua Shan, Sept. 1933 (G. Liu); Kwangsi\*: Pingloo, May 1933  
 (G. Liu); Szechuan\*: Suifu, alt. 1,000-1,500 ft., Oct. 10-26, 1930 (D. C. Graham).

**GENUS EURYSTYLUS** STAL**Key to Species**

1. Body above principally dark, pronotum with posterior margin distinctly sinuate ..... 2

Body above principally pale, pronotum with posterior margin nearly straight ..... *luteus*

2. Pronotum posteriorly with two black round spots surrounded with pale and a slender black median line, scutellum with basal angles and apex pale ..... *coelestialium*

Pronotum not marked as above, scutellum only with apex pale.....*costalis*

**EURYSTYLUS COELESTIALIUM** (Kirkaldy)

1902 *Olympiocapsus coelestialium* Kirkaldy, Trans. Ent. Soc. London, 1902, p. 255,  
 t. 6, f. 17-18.

1908 *Euryctyrtus bioculatus* Reuter, Ann. Mus. Zool. St. Pet., xii, p. 495.  
 Anhwei: Taipingshien, Oct. 1932 (G. Liu); Hopei: Peiping.

**EURYSTYLUS COSTALIS** Stal

1870 *Euryctybus costalis* Stal, Öfv. Sv. Vet. Aka. Förh., No. 7, p. 671.  
 Hopei\*: Peiping, July 1932 (G. Liu).

**EURYSTYLUS LUTEUS** Hsiao

1841 *Euryctyulus luteus* Hsiao, Iowa State College Jour. Sci., xv, No. 3, p. 247.  
 Anhwei.

## GENUS PHYTOCORIDEA REUTER

## PHYTOCORIDEA DISPAR Reuter

1906 *Phytocoridea dispar* Reuter, Ann. Mus. Zool. St. Pet., x, p. 22.  
Szechuan.

## PHYTOCORIDEA DISPAR var. DISCOIDALIS Reuter

1906 *Phytocoridea dispar* var. *discoidalis* Reuter, Ann. Mus. Zool. St. Pet., x, p. 23.  
Szechuan.

## GENUS TINGINOTUM KIRKALDY

## TINGINOTUM sp.

Szechuan\*: Tseo Jia Geo, alt. 3,000 ft., Sept. 23, 1929, Suifu, alt. 1,000-2,000 ft., Sept. 15, 1929 (D. C. Graham). The two specimens at hand have been so damaged that the antennae and legs are all gone and specific identification is very difficult. However, is it interesting to have the first record of the genus from China.

## GENUS PHYTOCORIS FALLEN

## Key to Species

1. Body clothed with pale pubescence; antennae pale yellow, last two segments slightly darker..... *sinicus*  
Body clothed with pale and black pubescence; antennae chiefly dark, spotted with white or green..... 2
2. Body above fuscous, conspurcate and varied with conspicuous green areas ..... *knighti*  
Body otherwise colored..... 3
3. Antennal segment I in length equal to length of pronotum, clothed with pale rigid hairs; sexes similar..... *intricatus*  
Antennal segment I in length greater than length of pronotum, clothed with black rigid hairs; male and female dissimilar..... *potanini*

## PHYTOCORIS INTRICATUS Flor

1860 *Phytocoris intricatus* Flor, Rhynch. Livil., i, p. 603.  
Szechuan.

## PHYTOCORIS SINICUS Poppius

1915 *Phytocoris sinicus* Poppius, Ann. Mus. Nat. Hung., xiii, p. 9.  
Kiangsu.

## PHYTOCORIS POTANINI Reuter

1906 *Phytocoris potanini* Reuter, Ann. Mus. Zool. St. Pet., x, p. 4.  
Szechuan.

## PHYTOCORIS KNIGHTI Hsiao

1941 *Phytocoris knighti* Hsiao, Iowa State College Jour. Sci., xv, No. 3, p. 249.  
Szechuan.

## UNCERTAIN SPECIES

## CAPSUS SINICUS Walker

1873 *Capsus sinicus* Walker, Cat. Hem., vi, p. 120.  
Hongkong.



# THE USE OF THE FUNGI IN MODERN GENETICAL ANALYSIS

CARL C. LINDEGREN

*From the Department of Bacteriology, University of Southern California*

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## INTRODUCTION

The fungi are pre-eminently suited to genetical experiments, but have been exploited to this end much less than *Drosophila* and maize. The reason is that they are too different from higher plants to permit direct transfer of *Drosophila* and maize techniques to them. The present paper will discuss the problems arising from these differences and show how these problems can be overcome. Because our work has been primarily with *Neurospora*, it is the example chosen in most cases, but similar problems arise in the genetics of the other fungi as well.

## MULTICELLULAR PLANTS

The higher plants are multicellular, and each diploid nucleus is enclosed within a single cell. These cells are the building blocks that make up the structures by which the organism performs its vital functions. The stability of the building blocks is assured by diploidy. A nucleus containing pairs of genes is safeguarded to a large extent against variation due to mutation because mutation of one member of a gene pair does not usually produce a variant (except in the rare case when the mutant gene is dominant); the normal gene is usually dominant over the mutant, and the phenotype of a cell made heterozygous by mutation is seldom altered. Gynandromorphs and chimeras are monsters, made up of mixtures of phenotypically different cells. They demonstrate the extreme necessity for genetically stable cells in the construction of a multicellular plant or animal, for many essential organs of gynandromorphs are malfunctional because of the phenotypical heterogeneity of the cells that compose them.

## COENOCYTIC PLANTS

An enormous evolutionary gap separates the diploid multicellular higher plants from the haploid fungi. Whenever a diploid nucleus is formed in a standard fungus life cycle, the number of chromosomes is thereafter immediately reduced in the usual manner to produce a tetrad. Diploid nuclei only occur in the zygote following karyogamy. There are only a few established cases in the higher fungi of diploid nuclei multiplying vegetatively.

A further fundamental difference between the fungi and the higher plants is that many of the fungi are essentially coenocytia. Although the hyphal threads of the ascomycetes are divided into chambers by

cross-walls, these septations are practically always perforate and function to keep the tubes from collapsing rather than to separate the organism into cells. The genetic mixture of haploid nuclei suspended in the common cytoplasm passes freely through the septations as the cytoplasm streams along the hyphal threads (3, 24, 25). If one of these haploid nuclei mutates, the mutant may continue to increase by division and become distributed throughout the plant by cytoplasmic streaming. The genetical heterogeneity of the nuclei prevents such an organism from producing structures of great complexity; in fact, these organisms seem to strive to maintain genetical complexity even at the expense of sacrificing the ability to produce complex structures. In some species, there are elaborate adaptations which assure that the sexual spores (which contain only a single type of nucleus in most species) will contain two genetically different kinds of nuclei (4, 7).

#### HYPHAL FUSION

Hyphal fusion or anastomosis is another device which functions to preserve and increase the genetical complexity of the fungi (3, 24, 25). When two hyphal tips come into contact their contiguous walls dissolve and their contents intermingle. After a hyphal wall becomes old and thick, it becomes incapable of fusion. These fusions have no sexual significance and are not followed by karyogamy. They occur between mycelia of the same sex as frequently as between mycelia of opposite sex. They sometimes occur between genetically different hyphae and may even occur between hyphae of different species or genera.

#### HETEROKARYOSIS

The facility of nucleo-cytoplasmic interchange produces a mixture of genetically different haploid nuclei. This condition is called heterokaryosis (30, 34, 41). When masses of spores are transferred the young germ tubes fuse with one another as soon as they emerge from the spores, and when the spores are genetically different, the new mycelium contains a mixture of different kinds of haploid nuclei. An interesting result of this capacity to produce nuclear mixtures is the fact that lethal genes in haploid nuclei can be carried in culture because the normal nuclei in the cytoplasm provide a sufficient excess of the materials necessary for subsistence. The fact that the "weaker" pseudo-parasitic or even lethal mutant nuclei can be supported by the more vigorous normal or wild-type nuclei means that practically all new mutations are preserved and live in the cytoplasm elaborated by the vigorous normal hosts. Since a gene is only "poor" or "good" in certain combinations, the low vigor of a genotype does not mean that the gene itself is not valuable for the species. The new genotype multiplying passively in the host cytoplasm recombines with other mutants in the sexual cycle. If a genotype of exceptional vigor or specific adaptability is eventually produced, it may attain supremacy in the heterokaryon and finally produce sectors of new

growth. Mutations in diploid cellular plants rarely attain supremacy in the plant in which they occur. Mutations which occur in somatic cells, even if they are dominant, are walled off from the rest of the plant and fail to affect its character unless they occur early in a bud (and cause a bud sport). An advantageous mutation in a fungus thallus, however, because of the coenocytic character of the plant, has a good chance of dominating the thallus although it can rarely become completely separated under natural conditions from the mixture of less vigorous nuclei from which it arose.

Some of the diverse nuclei found in fungus thalli are capable of performing specific functions. For example, in *Neurospora*, forms found in nature may be heterokaryotic for normal conidial and nonconidial mutant nuclei. These nonconidial mutants are incapable of producing the asexual spores (conidia) by which the fungus is disseminated vegetatively. Since they do not expend energy in the formation of asexual spores, they grow more rapidly than the normal conidial forms. The heterokaryon has the rapid growth character of the nonconidial mutant and the conidium-forming capacity of the conidial form and is, therefore, more vigorous than either pure form. It is conceivable that mixtures of nuclei are important in other ways not so easy to demonstrate. A heterokaryon carrying mutants capable of elaborating different kinds and amounts of enzymes would probably have a higher survival value than a more restricted and less flexible homokaryotic form. In bacteria this may be an extremely important factor in the survival of the species, especially in the special case of the "free cell" structure of the bacteria.

Organisms such as fungi and bacteria, in which single thalli or colonies contain mixtures of genetically differentiated nuclei can only possess a minimum of differentiated structures and in place of structures capable of performing a variety of functions genetically, different nuclei may each be specifically adapted for the performance of different functions. In higher plants which are genetically uniform and protected against variation by diploidy, different functions are performed by structures differentiated morphologically from the other cellular tissue of the organism. Fungi which produce relatively complex structures such as the Hymenomycetes are built of cells each of which contains a stable dikaryon which is duplicated by synchronous "conjugate division" of each of the nuclear mates. In mushrooms each cell contains two nuclei and this pairing insures stability against the possibility that mutation might produce a new phenotype and distort the structure. Stability is, therefore, assured by dikaryons in Hymenomycetes just as it is assured by diploidy in the cells of higher plants. However, a hymenomycete dikaryon is an extremely flexible structure, and one of the members of a dikaryon is often replaced by an "invading" nucleus of the same sex but possibly genetically different. Dikaryons are technically quite different from diploid nuclei and Buller's term "diploidization" is not accurate. He uses this term to describe the process of converting a haploid mycelium

into a dikaryotic mycelium. Strictly speaking "dikaryotization" would be a preferable term. Smut mycelia capable of invading host tissue and causing disease are dikaryotic. Monokaryotic strains of smuts are usually incapable of parasitism while the dikaryotic bisexual mycelium easily gains entrance to the host tissue.

#### ASEXUAL CYCLE

Asexual spores (conidia) of *Neurospora* are usually multinucleate, thus insuring the vegetative propagation and dissemination of the heterokaryon existing in the thallus (6, 29). When a conidium is finally separated from the conidiophore by a solid wall, it may contain a few to a dozen or more haploid nuclei. In nature the variety of mutant nuclei present in a single conidium usually contain representatives of each of the two sexes. A conidium planted on agar produces a germ tube which branches rapidly and grows radially from the point of inoculation with the concomitant multiplication of the various nuclei.

The mycelium is made up of branching tubes divided into cylindrical compartments by septa (cross-walls) but these cross-walls do not separate the threads into cells in the regular sense. Two partitions may enclose a mass of cytoplasm containing any number from a few dozen to many hundreds of nuclei, each of which is haploid. Furthermore, each septum has a large perforation at the center, and nuclei migrate freely through the perforations. When the hyphae have attained nearly full growth, an abundant deposit of nuclei and cytoplasm has accumulated. During the early stages of growth only a few conidia are produced, but finally, when the growth of the hyphal mat nears its limit, conidia are formed in abundance.

The large hyphal tubes in the center of the mat are completely emptied of their nucleo-cytoplasmic mixture, and as the emptying progresses, a plug is formed at each septal perforation. (Sometimes a thin hyphal thread will grow back into the large emptied hypha through the perforation.) The abundance of already elaborated cytoplasm and nuclei is stuffed like the filling of a sausage into the terminal hyphae, which now takes on a predominantly aerial growth, probably stimulated by the staling of the substrate. The aerial hyphae branch dichotomously, and the conidia are cut off by walls as the streaming into the tips continues.

#### SEXUAL CYCLE

The sexual spores of *N. crassa* are black, lemon-shaped ascospores with longitudinal markings (37, 42). After a heat treatment at 60° C. for an hour or 90° C. for a shorter time, the ascospores put out germ tubes from pores at each end of the spore. In nature the spores lying dormant in the ground usually germinate after a fire. Before any green vegetation gets started the aerial hyphae covered with salmon pink conidia (asexual spores) cover the burnt-over region. The mold sometimes completely obscures the object on which it is growing. The growth

is so thick that conidia can be picked up by handfuls from the surface of bread or sugar cane bagasse. Slight disturbances of the air release clouds of conidia to spread the infection. Finally ascospores are produced as a result of the sexual cycle and the ascospores may lie dormant in the soil for several years until the next fire occurs. The asexual spores usually die in a few months.

In the laboratory, it is possible to obtain pure cultures from the ascospores of *N. crassa* by planting them on agar and subjecting them to a heat treatment (26). This heat treatment kills the vegetative mycelia and asexual spores and stimulates the ascospores to germinate. After the ascospores germinate, a small block of agar containing a single spore is cut out and transferred to a culture tube. Such cultures contain an abundance of vegetative mycelia and asexual spores, but no ascospores appear in a culture grown from a single ascospore. The fungus is heterothallic, producing (+) and (-) ascospores. Cultures from either (+) or (-) spores look exactly alike to the naked eye and under the microscope. When (+) and (-) mycelia are planted together in a tube, perithecia filled with ascospores are produced (27).

The male gametes are called spermatia (1, 11, 14, 21, 46). They are produced on both (+) and (-) mycelia. The spermatia are small ( $3\text{-}4 \mu$ ), thin-walled, uninucleate cells which act as sperm. They are produced by being extruded from a pore at the tip of a bell-shaped hypha or from pores in the sides of short stubby hyphae (spermatiophores). Plus spermatia only fertilize (-) cultures, and (-) spermatia can fertilize only (+) cultures.

The bubils are the female sex organs. Each contains an oogonium tightly wrapped in a dense ball of thick-walled hyphae. From the oogonium a branched trichogyne extends sometimes for a long distance. Plus bubils are transformed into perithecia containing asci full of ascospores if they are spermatized by spermatia from a (-) culture. Bubils which are indistinguishable from each other are found in both (+) and (-) cultures.

#### SEGREGATION IN THE ASCUS

The ascospores are contained in the slender tubular asci inside the perithecium. From 50 to 100 each are normally found in each perithecium, and each ascus contains 8 spores. Each ascospore is binucleate, but the ascospore at its origin contains only a single nucleus, so these nuclei are genetically identical and the culture produced by growing a single spore is homokaryotic. Four of the spores from each ascus are (+) and four are (-) (27). The zygote is produced by a nuclear fusion occurring in the young ascus. One haploid (+) gametic nucleus and one haploid (-) gametic nucleus always participate in the fusion. The zygote immediately undergoes reduction, and the distribution of the two types of spores in the ascus is due to the reduction of a  $+/-$  gene pair in the zygote. The fact that (1) the zygote is always heterozygous for the  $+/-$

gene pair, (2) that no zygote can be produced unless it is heterozygous for these genes, and (3) that every member of the species falls into either the (+) or (-) category with no alternative class, establishes these genes as sex factors rather than sterility factors. Sterility factors are well known in *Neurospora* and have been extensively studied, but cannot possibly be confused with the sex factors. We have, therefore, two sex mechanisms involved in producing the zygote: (1) the sex organs, spermatia (male) and bulbils (female), and (2) the sex factors, (+) and (-), for which the zygote is always heterozygous. The two kinds of spores are arranged in the ascus in six ways:

Arrangement	SPORES							
	1	2	3	4	5	6	7	8
(1)	+	+	+	+	-	-	-	-
(2)	-	-	-	-	+	+	+	+
(3)	+	+	-	-	+	+	-	-
(4)	-	-	+	+	-	-	+	+
(5)	+	+	-	-	-	-	+	+
(6)	-	-	+	+	+	+	-	-

The first two arrangements (four and four) are the result of Mendelian segregation of the sex factors at meiosis I. The last four arrangements (two and two) are the result of Mendelian segregation of the sex factors at meiosis II.

The third division is equational, so each spore pair (1 and 2, 3 and 4, 5 and 6, 7 and 8) is genetically identical. A fourth division occurs in each spore which is also equational, and results in each spore containing a pair of genetically identical nuclei.

The ratio of first-division segregation to second-division segregation of the sex genes is constant and equals 87:13. The second division segregations are due to cross-overs occurring between the locus of the +/— gene pair and the centromere (28). This ratio determines the distance of the sex genes from the centromere.

Segregation of other mutant genes from their normal alleles is regular and occurs with constant ratios of first- to second-division segregation. When two mutants are mated the complexity of spore arrangement is increased; there will be  $(6 \times 6) = 36$  possible types of arrangements in the ascus (33).

Many experiments in fungus genetics, especially with rusts and smuts, have been interpreted to indicate that the reduction division is irregular. That is, the reduction of a nucleus heterozygous for a pair of genes does not always produce a 1:1 ratio of variant to normal. It has already been stated that, with regard to the pair of sex genes, the ratio of (+) to (−) is always 1:1. This is also true of all the other mutant genes studied in *Neurospora*. In complex crosses, i. e., when the zygote is heterozygous for three or more genes, analysis is extremely difficult due to the difficulty of determining the genotype of the progeny by simple inspection. This is due to a number of factors, (1) modifiers, (2) epi-

stasis, (3) phenotypically identical genotypes, and other difficulties to be discussed later. Cases reported in smuts and rusts of irregular segregation are probably only superficially irregular. The irregularities in segregation would probably disappear if each of the variants were mated to a wild type stock and their progeny analyzed. Continued mating of progeny to wild type has not been established as a standard practice in smut genetics, and yet it is essential to any final analysis. In the many thousands of asci which we have analyzed from hundreds of matings, the segregations have been regular. In cases of apparent irregularity, subsequent work always resulted in a clarification of the mechanism.

#### CHROMOSOME MAPS AND CHROMOSOMES

Chromosome maps can only be constructed by extremely intensive study of a single species. Much work on fungal variation has been done by mycologists and plant pathologists who spread their studies over a wide variety of species. The monumental success of Bridges in constructing chromosome maps of *Drosophila* was due to a lifetime of the most intensive effort on the single species with which he began. His work has been supplemented by hundreds of other investigators and the maps of *Drosophila* chromosomes are the result of many lifetimes of effort directed at this single species. Twelve years of work on *N. crassa* alone were required to construct the maps of two chromosomes: (1) The sex chromosome with six loci: *sex*, *gap*, *centromere*, *crisp*, *pale*, and *dirty* (35). (2) The second chromosome with six loci: *centromere*, *ring*, *peach*, *tuft*, *burnt*, and *fluffy* (39). Many other genes have been studied, but not definitely located in linkage groups.

Cytological study indicates that there are probably six chromosomes in *Neurospora crassa* (38). Dr. McClintock and Edward Weaver have shown preparations of *N. sitophila* in which they found seven chromosomes. The six chromosomes of *N. crassa* are of unequal length. Study of leptotene shows the following approximate number of chromomeres in each of the respective chromosomes: 29, 18, 13, 9, 5, and 3.

#### HOMOKARYOTIC STRAINS

The first step in genetic study of the fungi is to establish and maintain pure homokaryotic cultures (28). This essential rule is usually not observed by students of fungal variation. Much work has been done using polysporous transfers containing both conidia and ascospores. Some workers think that cultures which have been carried for years in the laboratory are especially desirable for studying variation because they have become "stabilized." Such cultures are not stabilized because they are homokaryotic but because they have become adjusted to artificial media by mutation, thus establishing the supremacy of a certain genotype-group, and associated with this genotype-group is invariably a large assemblage of pseudo-parasitic, semi-lethal, or "weak" nuclei carried in the common cytoplasm at the expense of the vigorous forms

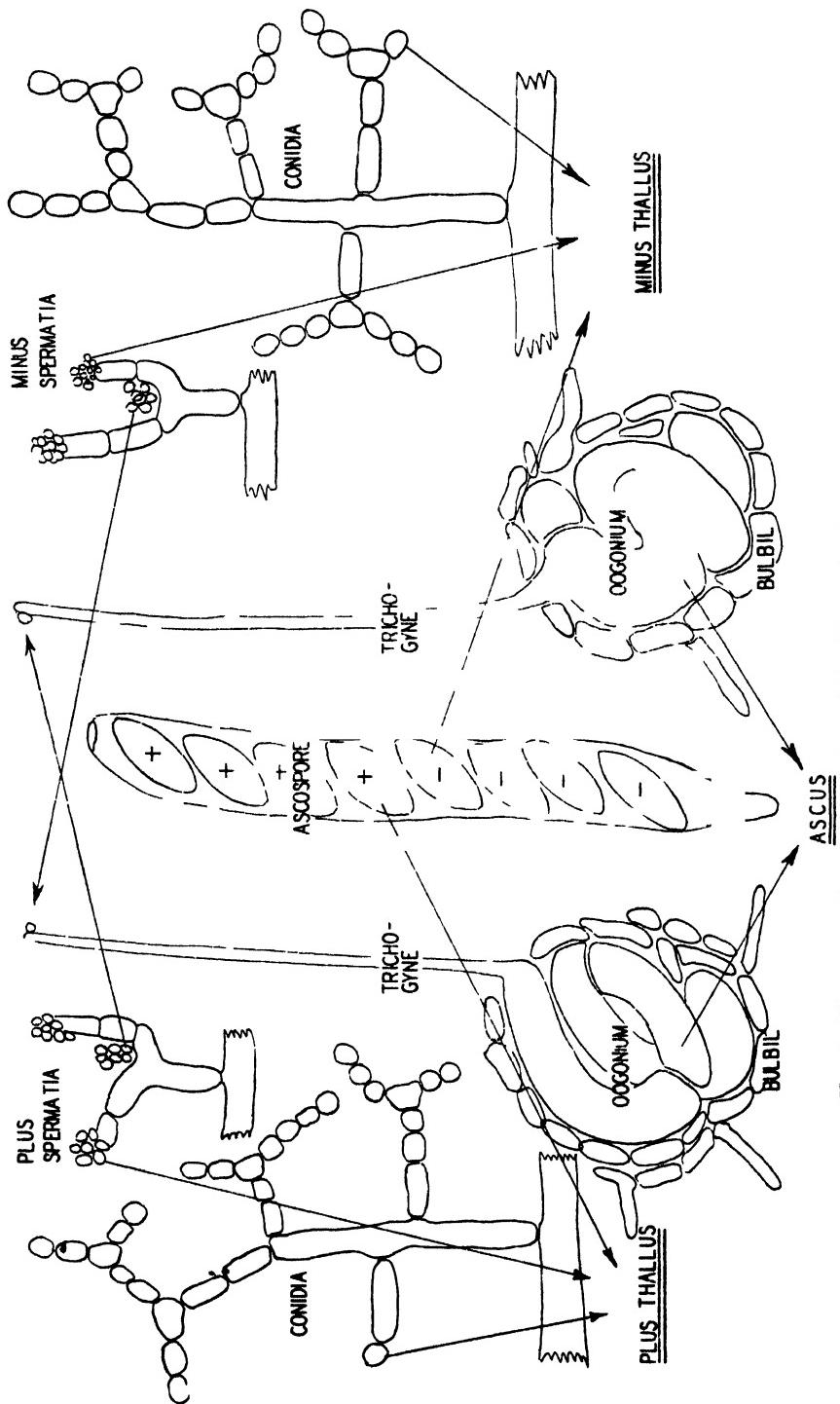


FIG. 1. Diagrammatic representation of the life cycle of *Neurospora crassa*.

(30). In our work we have analyzed many mixtures of this type in *Neurospora*. *Neurospora* cultures which have been propagated vegetatively for six months or more are always heterokaryotic, and one of our standard laboratory routines is the purification of these cultures before every genetical test. The mutations present are usually not evident by inspection but can be separated by various techniques (33).

#### PRODUCTION OF MUTATIONS IN FUNGI

Because the fungi are so variable, many studies of the factors which influence this variability or "mutability" have been made. Unpurified stock fungus cultures are subjected to different treatments, and a variety of "new" forms are obtained. The fundamental error in these experiments is that the treatment is assumed to have produced a change in the genotype without consideration of the alternative possibility that the variants already present were merely separated from the mixture by treatment. Most experiments describing the "production" of fungus mutations are probably in error in assuming that the agent used to "produce" the mutation actually caused the mutation to occur.

In our hands most fungus genes have proved to be as stable as the genes of either maize or *Drosophila*. The frequency of occurrence of heterokaryotic cultures is consistent with this statement when one considers (1) the large population of nuclei and the possibility that any vigorous mutant may obtain supremacy and (2) the various devices in the fungi which encourage the development of heterokaryotic thalli in contrast to the higher plants and animals which are purified genetically each sexual generation. The occasional highly mutable genes found in *Neurospora* mutated independent of the environment, and the spontaneous mutants seemed to occur independent of the environmental conditions (29). It is possible that the so-called "mutation-producing" agents merely separate different genotypes already present in a heterokaryon rather than produce new mutations. In these experiments the type of culture used is more important than the agent which indicates that the culture is impure rather than that the agent is effective. Agents whose efficacy has been demonstrated in *Drosophila* and maize, like ultraviolet and X-rays, produce mutations with equal facility from all types of cultures.

#### TECHNIQUES FOR SEPARATING HETEROKARYONS .

The spermatia which function as sperm to fertilize the oogonia can also reproduce asexually (11). A single spermatium planted on agar will produce a germ tube and eventually a thallus. The spermatium is uninucleate, and a single spermatium culture is homokaryotic. This is our present technique of purifying *Neurospora* cultures or of analyzing the complexity of a heterokaryon. Single ascospore cultures are also homokaryotic.

It is possible to separate the different components of a heterokaryotic

culture by mating it to normal, and culturing single ascospores obtained from the mating. This method succeeds because each ascospore contains only a single nucleus at its origin. Asexual subculturing of single conidia usually fails to effect a separation because these asexual spores are multinucleate and nearly as heterokaryotic as the original thallus.

#### VARIETY OF MUTANT FORMS

Normal cultures of *N. crassa* produce an abundant growth of salmon-pink conidia borne on a well-developed system of aerial hyphae. The conidia are borne in chains (catenulate) which branch dichotomously. The conidiophores are divided by septations into short, compact oidia. Mutants are all identified by alterations of this standard type of cultural growth. Many mutants known to be due to different genes resemble each other so closely that they are indistinguishable.

The nonconidial mutant is the one most frequently encountered. In this type of mutant, the production of salmon-pink conidia is suppressed, and a thick, white growth of aerial mycelia takes its place. Spermatia and bulbils are especially abundant, and these mutants are vigorous and very fertile. They also grow more rapidly than normal because less energy is expended in producing conidia than in the normal form. *Fluffy*, *webby*, *cottony*, *albinistic*, and *soft* fall into this category.

An analysis may be complicated by confusion between different variants which are indistinguishable except through genetical tests. This is true of the nonconidial mutants. The technique of solving this difficulty is described below.

*Gap* resembles the nonconidial forms in producing a few conidia at the upper fringe of a mass of nonconidial growth. *Pag* produces a few clusters of conidia at the base after first putting out an abundant nonconidial growth. Both these forms are characteristically vigorous and fertile, thus resembling the mutants in the nonconidial category.

*Pale*, *peach*, and *tuft* produce fewer conidia than normal and show generally reduced vigor. They are lighter colored than normal, probably because they produce fewer conidia.

*Crisp* and *fan* are characterized by an exceptionally abundant production of conidia, but the conidia are clustered close to the agar. These forms might be considered to be at the opposite extreme from the nonconidial types, for they produce an excess of conidia with a minimum of aerial supporting hyphae.

*Dirty* and *even* are characterized by a deformity of the conidia. The conidia are large globoid, and are neither dichotomously branched nor catenulate. A reduced growth of white conidiophores is topped by a growth of these deformed conidia and relatively abundant accumulation of a yellowish "dew" collected on the hyphae. This "dew" is possibly an exudate.

*Tan* differs from normal by a considerable reduction in both conidia and mycelia. The substrate appears to have a buttery sheen and a tan, leathery color. This characteristic substrate color is found in many vari-

ants of markedly reduced vigor. *Tan* has the additional distinction of being a rapidly mutating gene. The first transfer produces a culture resembling normal, but genetic tests readily show that the culture is heterokaryotic, containing both *tan* and normal nuclei. The *tan* nuclei mutate to normal with relatively high frequency.

*Black* is a character of the substrate. A black mutant differs from normal in producing a dark band at the upper edge of the substrate. Attempts to use the black substrate color mutants in genetic experiments have been generally unsuccessful because the phenotype is extremely variable. Black substrate is especially pronounced in cultures grown on rich medium or kept in bright sunlight. *Melanistic* also falls into this category. This type of variant segregates relatively frequently from interspecific crosses. It shades almost imperceptibly into the *tan* category, and like it, seems associated with a certain degree of degeneration.

The sex locus is a valuable one because it can be superimposed on any other gene or combination of genes, since it produces no phenotypic effect except capacity to mate with the opposite sex. This is important because most combinations are less vigorous than single gene mutants and the number of genes that can be mated in a single cross is limited. The more genes a mutant carries, the harder it is to determine its genotype by inspection, because each additional gene reduces the vigor until, finally, too small an amount of growth is produced to afford a basis for diagnosis. The sex gene does not affect the vigor of a combination in which it appears.

#### CLASSIFICATION OF PROGENY BY ELIMINATION

In *Drosophila* and maize, the differentiation of the organism makes it possible to find genes which specifically affect different structures. For example, white is a gene which changes the normal red eye to white and vestigial is a gene which changes the normal long wing to a short vestigial structure. The double mutant (white vestigial) has white eyes and vestigial wings. Neither gene interferes with the expression of the other seriously enough to make classification difficult. In *Neurospora*, the simple structure of the plant and the absence of differentiated structures means that practically every gene affects the entire thallus and, therefore, has an effect on the expression of every other gene. For this reason, the phenotype of a double mutant is not predictable. However, the double mutant can be selected from the progeny by elimination especially when all eight spores are grown from an ascus. A cross of *pale* (light salmon, conidial) by *fluffy* (nonconidial) produces two types of new combination progeny. These are (1) nonpale nonfluffy (normal) and (2) *pale-fluffy* (the double mutant) (28). Three types of ascci, with respect to their mutant combinations are found:

1 P	2 PF	3 PF	4 PF	5 nor	6 nor	7 nor	8 nor	original only new combination only original and new combination
P	PF	PF	PF	nor	nor	nor	nor	
PF	PF	P	P	F	F	nor	nor	

Other types of ascospores are found with respect to the different arrangements of the original and new combination genotypes in the ascus. Three of the genotypes, *pale*, *fluffy*, and normal, can be easily classified from earlier experiments. If a single ascus contains the four normal cultures, the remaining four must by elimination be the double mutants, *pale-fluffy*. This follows from the fact that the genes are always segregated in the ascus in a 1:1 ratio, and if four of the spores receive the normal allele of both mutant genes, the other four spores must have each received both mutant genes. A pair of *pale*, a pair of *fluffy*, and a pair of normal cultures from a heterozygous ascus means that by elimination the fourth pair of cultures must necessarily be the *pale-fluffy* double mutant. The *pale-fluffy* double mutant does not resemble either single mutant parent, so its phenotype is no guide to its genotype. It is a grey, much reduced growth, without either the light orange color of *pale* or the white, abundant growth of *fluffy*. However, a mating of the double mutant to normal produces the same classes of progeny as a mating of *pale* by *fluffy*, thus proving its genotype to be *pale-fluffy*.

#### ABERRANT SEGREGATION RATIOS

##### A. MODIFIERS

Aberrant segregation ratios may result from the interaction of two or more genes in producing the phenotype (28, 29). This is especially clear in the case of genes modifying the nonconidial mutants. When the nonconidial mutant *fluffy* is mated to normal, the four normal and four nonconidial cultures are seldom exactly alike. Two of them are usually slightly different from the other two. This is because the normal form carried a gene capable of modifying *fluffy*, but this modifying gene did not produce a visible effect on normal. Rapidly growing mutants like the nonconidial types are extremely susceptible to modifiers which may have no visible effect at all on the phenotypes of the slower growing conidial mutants. Modifiers interfere with genetical analysis as the following example will demonstrate: A mutant (slow-growing conidial) *pale* stock was inbred for six generations and mated to mutant, *fluffy* (nonconidial), stock which had been similarly inbred for six generations. This amount of inbreeding was expected to purify the stocks so that crossbreeding of *pale* by *fluffy* would yield only four types of progeny: (1) the two original combinations, *pale* and *fluffy*, and (2) the two new combinations *pale-fluffy* and normal. However, five types of progeny were obtained; one of them was a modified *fluffy*. Apparently the *pale* line was homozygous for a gene which modified *fluffy* and which produced no phenotypic effect on *pale*. In such cases, the *pale* stock is purified by selecting a *pale* culture from an ascus of the following types:

Spore Genotype	1 P	2 P	3 P	4 P	5 F mod	6 F mod	7 F mod	8 F mod
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Since the four *fluffy* spores all carry the modifier, it must be absent

from the *pale* cultures. When purified, the *pale* cultures are crossed with unmodified *fluffy*, only four types of progeny are obtained.

#### B. GENE COMBINATIONS OR MULTIPLE MUTANTS

Combinations of genes in a single haploid nucleus do not usually give predictable results. In some combinations, one gene may produce the preponderant effect on the phenotype. For example, in most combinations carrying *crisp*, the *crisp* effect is obvious while other genes in combinations with *crisp* may be difficult to detect. *Crisp-pale* is slightly lighter than *crisp*, but *gap-crisp* resembles *crisp* so closely that unless one were completely familiar with this double mutant, he would mistake it for *crisp*. It differs only in a yellow substrate due to the presence of the *gap* gene.

Sometimes neither component is recognizable, *pale-fluffy* looks like neither *pale* nor *fluffy* and is not what one would predict the combination ought to look like. This type of effect is rather common.

A double mutant is practically always less vigorous than a single mutant. The only exception encountered is the *gap-clump* double mutant. *Clump* is a modifier of *gap* detectable only when in combination with *gap* (33). This combination is always more vigorous than either *gap* or *clump* alone. *Gap-clump* produces more conidia and grows more vigorously than normal.

Since gene combinations are not always predictable or detectable, the progeny from a mating may not give what appears to be regular ratios until further analysis is made by mating the new forms by wild-type.

#### C. PHENOTYPE OF HETEROKARYONS

The above discussion concerned combinations of genes in a single haploid nucleus to form a homokaryotic thallus. A heterokaryotic thallus is a gene combination in a different sense and often assumes the character of the more rapidly growing component with few or no sectors indicating the presence of the fellow-traveler. If one of the components of a heterokaryon is normal, the entire thallus is usually phenotypically normal. Mating a mycelium which is assumed to be homokaryotic but which is actually heterokaryotic will result in an odd assortment of sexual progeny.

#### D. EPISTASIS

Epistasis also produces irregular ratios. We have already discussed some phases of the problem of epistasis in pointing out that *gap-crisp* shows little phenotypic evidence of the presence of a *gap* gene. This is only partial epistasis, for it is possible by critical study to detect the presence of *gap* in the combination. However, there are cases of apparently complete epistasis (29). *Pale* is epistatic to *tan*, so that the double mutant *pale-tan* shows no sign of the presence of *tan*, although *tan* is a

very conspicuous phenotype. For example, crossing *pale* by *tan* produces many ascospores of the following type:

Spore	1	2	3	4	5	6	7	8
Phenotype	pale	pale	pale	pale	tan	tan	nor	nor
Genotype	PT	PT	P	P	T	T	nor	nor

Genetic analysis of *pale* cultures from spores 1 and 2 revealed them to be *pale-tan* double mutants. Mating the mycelium from these ascospores with a normal mycelium results in the appearance of many *tan* progeny definitely proving the epistatic effect of *pale*.

#### E. PHENOTYPICALLY IDENTICAL GENOTYPES

The various nonconidial mutants all resemble each other very closely, and the most critical examination of cultures of the same age and on the same substrate seldom reveals a real distinction. They can be shown to be different, however, by a genetic test. This test is especially important in studying fungal variation because it serves to distinguish phenotypically identical variants from each other. When the two nonconidial variants, *fluffy* and *albinistic*, are mated, three kinds of ascospores are obtained with respect to the types of progeny produced (not the arrangements of spores) (*F* = *fluffy*; *A* = *albinistic*; *nor* = normal (conidial); *non-c* = nonconidial; and *cn* = conidial).

Spores	1	2	3	4	5	6	7	8	Ratio Non-c to cn
Genotype	F	F	F	F	A	A	A	A	4 : 0
Phenotype	Non-c								
Genotype	FA	FA	FA	FA	nor	nor	nor	nor	1 : 1
Phenotype	Non-c	Non-c	Non-c	Non-c	cn	cn	cn	cn	
Genotype	F	F	A	A	FA	FA	nor	nor	3 : 1
Phenotype	Non-c	Non-c	Non-c	Non-c	Non-c	Non-c	cn	cn	

Other arrangements of spores are encountered; only three are indicated. The outstanding fact is that two apparently identical nonconidial phenotypes when mated produce some normal conidial progeny. This is explained by the fact that *fluffy* carries the normal allele of *albinistic* at the *albinistic* locus in addition to the *fluffy* gene at the *F* locus. *Albinistic* carries the normal allele of *fluffy* at the *fluffy* locus. Representing the mutant genes by capitals and their normal alleles by small letters, the *fluffy* and *albinistic* stocks will be designated respectively: *Fa* and *fA*. When these genotypes are mated four types of progeny: (1) *Fa*, (2) *fA*, (3) *FA*, and (4) *fa*, are expected. The *fa* cultures are normal. The double mutant *FA* cultures are phenotypically indistinguishable from either pure *fluffy* or pure *albinistic*. Attempting to analyze the progeny from such a cross, without knowledge of the fact that the nonconidial phenotypes constitute three different genotypes, may lead to the belief that reduction has occurred irregularly in some ascospores in which the ratio of nonconidial to conidial is 3:1.

However, genetical analysis shows that the underlying ratios are actually 1:1 gametic ratios. Four mutant and four normal genes always segregated at each meiosis.

#### F. RAPIDLY MUTATING GENES

The first mutant encountered in the genetical analysis of *Neurospora crassa* was *tan* (29), which is an unstable, rapidly mutating gene. It mutates from *tan* to normal, and a pure *tan* culture very soon becomes heterokaryotic by mutation. The first transfer of a *tan* culture to a fresh tube of medium produces a culture resembling normal due to the fact that many of the nuclei present in the heterokaryon are normal, and these rapidly growing nuclei provide a cytoplasm which nurtures the *tan* nuclei and at the same time prevents the *tan* phenotype from appearing. *Tan* cultures can be recovered by making subcultures of hyphal tips, proving that many of the nuclei in the normal-appearing heterokaryon are *tan*. When the heterokaryotic (apparently normal) cultures were mated, the zygote obtained either *tan* or normal nuclei from the heterokaryotic culture with the result that some of the asci produced by mating this apparently normal heterokaryon by a true normal mycelium contained four *tan* spores and four normal spores, while the other asci contained eight normal spores.

A cross of *tan* by *tan* also produces many normal progeny, because each *tan* mycelium contains normal nuclei produced by mutation. Some of the normal progeny of these matings produce *tan* progeny, indicating that suppressors of *tan* are present which make the double mutant (*tan* and *tan-suppressors*) look like normal, thus adding a complication. This discussion shows that with rapidly mutating genes, unpredictable ratios are the rule. These crosses are extremely confusing and require extended analysis.

Many of the confusing and apparently non-Mendelian results obtained in analysis of smuts and rusts are due probably to the use of stocks carrying unstable rapidly mutating genes. This is indicated by the description of the sectoring of monosporidial cultures. However, most genes in fungi are stable, and unstable, rapidly mutating genes are encountered as frequently in corn and *Drosophila* as in the fungi.

#### G. COMPLEX CROSSES

A mating heterozygous for two genes produces four types of progeny; one heterozygous for three genes produces eight types of progeny, and when the mating is heterozygous for four genes, 16 types of progeny appear. If a mating is made between a multiple mutant stock which is assumed to be a single mutant or between a heterokaryotic stock which is assumed to be homokaryotic, much difficulty is encountered in making the analysis. Both problems often arise simultaneously. For example, a single ascospore culture which was a markedly clean-cut mutant was

mated by a supposedly homokaryotic normal stock. This normal stock was actually heterokaryotic for *peach*, *clump*, and *normal* (33). The single ascospore mutant culture was actually a double mutant, *gap-dirty*. Fifteen types of progeny were obtained when only two were expected. An entire summer was required, but the analysis was successfully completed and confirmed. The most important factor in making the analysis possible was the knowledge that any given mutant is always segregated with a fundamental 1 : 1 ratio so that in any ascus half of the spores carry the mutant gene and half carry its normal allele.

#### H. COMPETITION WITHIN THE ASCUS

A peculiar complication is developmental competition between different genotypes within the ascus (32). When a zygote is heterozygous for a gene which produces a very rapidly growing phenotype and also heterozygous for a gene which produces a very slowly growing phenotype, the vigorous phenotypes may ripen ahead of the slowly growing phenotypes. This may result in only four spores ripening because the slowly ripening spores have been deprived of nutrients. An example follows: both *pale* and *smooth* are slow growers, while *fluffy* stocks are extremely vigorous and rapid growers. When *pale-fluffy* was mated to *smooth*, segregation produced some asci containing the four genotypes *fluffy*, *pale-fluffy*, *smooth*, and *smooth-pale*. In such an ascus only the *fluffy* spores ripen, and the others are too slowly developing to obtain nutrients to enable them to ripen. In these asci only the two *fluffy* spores turn black, while the other six are colorless (aborted) and fail to ripen.

It is a routine practice to test for such competition by dissecting asci containing unripe spores.

#### ADVANTAGES OF NEUROSPORA

The short life cycle of *Neurospora* offers special advantages in genetic analysis, for mature progeny can be obtained in from two to three weeks after a mating has been made. These cultures can then be kept for many months and studied in every stage of development. They can be replanted on a variety of substrates for study under different environmental conditions. By transfer of conidia, as many copies of a single ascospore culture can be made as desired.

The expense of fungal genetics is much less than that of other forms of plant genetics. Practically the only cost is for test tubes and nutrients.

Since the plants are haploid, there is no obscuring effect of dominance and recessiveness. This is comparable to growing mature plants and animals from pollen grains or sperm. Each haploid ascospore has an identical twin which serves as a check on its phenotype.

The maintenance of stocks is very simple. Ascospores can be put away under the proper conditions of storage for an indefinitely long time and revived whenever needed. We have often recovered cultures that

have been stored without attention for as long as five years. Coenocytial structure and hyphal fusions open up the possibility of experiments on the effect of mixtures of different types of cytoplasms and nuclei.

#### TETRAD ANALYSIS

The collection of the four genotypes produced by the reduction of a single spore mother cell is most easily carried on with the fungi. This is especially important in the study of crossing-over because it eliminates the necessity of inference and permits direct observation of the genotypes of the four chromatids produced. Tetrad analysis with *Neurospora* has resulted in considerable alteration of rather well-established concepts concerning the mechanism of crossing-over. It was previously thought that exchanges between the four chromatids occurred at random, but it has been shown that these exchanges occur in a specific nonrandom manner in relation to the various regions concerned. The study of crossing-over by tetrad analysis has not resulted simply in confirmation of old and well-established principles, but has revolutionized the concept.

The fact that the reduction division occurs in a long, narrow tube, makes it possible to distinguish first from second-division segregation of any given pair of alleles. The spores lie in the ascus in six different patterns, two of which result from first-division segregation and four of which result from second-division segregation. The work on *Neurospora* conclusively established the fact that second-division segregation is the result of a cross-over occurring between the centromere and the locus of the allel in question (36).

#### X-RAY AND ULTRAVIOLET INDUCED MUTATION

The spermatia of *Neurospora* are well adapted to radiation experiments, for they are much smaller than pollen grains. A smaller proportion of incident radiation is absorbed by structures comparable to the wall of the pollen grain, because the walls of the spermatia are extremely thin; an adaptation to fit them to their primary function of fusing with the trichogynes of the bulbils. The uninucleate spermatia were obtained from the nonconidial variant which produces spermatia although it does not produce conidia. These nonconidial variants are very readily affected by the presence of modifying genes and, therefore, provide an excellent background for the detection of mutation. When a culture was obtained as a result of treatment, which was obviously different from the original nonconidial variant, it was mated to the wild-type tester strain, and four types of progeny were usually obtained corresponding to the double mutant, nonconidial mutant, the new mutant, and wild-type. Chromosomal aberrations were revealed by the abortion of certain ascospores in the ascus. Some ultraviolet mutations were found to be simple gene changes; some X-ray gene mutations were associated with chromosomal aberrations. In addition, ultraviolet induced degenerate phenotypes which did not yield to genetical analysis.

### SUMMARY

The use of the fungi for genetical experiments depends on understanding the nature of the plant structure. Although haploid inheritance is fundamentally the same as diploid inheritance, its manifestations are markedly different. Examples of some of these peculiar manifestations are given to clarify the problem. Understanding the problem makes a simple direct approach possible. The fruitfulness of the fungi in genetical analysis is shown by specific examples of their use in the study of crossing-over, and the induction of mutation.

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# LEVULOSE FROM CHICORY, DAHLIAS, AND ARTICHOKE<sup>1</sup>

E. S. HABER, W. G. GAESSLER, AND R. M. HIXON<sup>2</sup>

*From the Subsections of Vegetable Crops and Plant Chemistry,  
Iowa Agricultural Experiment Station*

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In order that levulose may be produced at a low price, it will be necessary that the raw product, agricultural crop, can be produced easily and at a low cost. The levulose content of a species of plant, if it is to be used as a commercial source, must be high. Some of the levulose-yielding plants include the dahlia, chicory, dandelion, Canadian thistle, goldenrod, grasses, Jerusalem artichoke, wild onion, etc. The low levulose content of some of the plants eliminates them from serious consideration as a commercial source of levulose. Propagation, cultivation, and harvesting problems eliminate others. The dahlia, chicory, and Jerusalem artichoke seem to be the most promising because of levulose content, and because the agronomic problems involved in their production are not too serious.

## AGRONOMIC PHASE

In 1939, dahlias, chicory, and artichokes were grown at Ames in loam and in Hancock County in deep peat soil. About 600 pounds per acre of superphosphate were applied to the soil at Ames for the three species. About 800 pounds per acre of an 0-9-27 fertilizer were applied to the peat soil. Tons per acre of chicory were greater from peat than from the loam. Dahlias produced about the same amount on both soils. Artichoke plants produced tubers about the size of marbles in the peat soil. No doubt the temperature of this deep peat was too low, so that the plants grew slowly and frost killed the "tops" while tubers were small.

In 1940, dahlias, chicory, and artichokes were planted in loam at Ames, but only the dahlias and chicory were planted in the peat since the artichokes failed to produce tubers in peat the year before. Without exception the levulose content was higher in chicory roots grown in the peat soil in both years. Although chicory is a biennial, some plants produce seed stalks the first season grown. Yield of levulose from such roots was equal or better in the Magdeburg variety than in nonblooming plants. In 1939 about 90 per cent of the Magdeburg variety produced flower stalks; in 1940 about 50 per cent of the plants produced flower stalks. In Fredonia and Silicion Half-Long varieties of chicory, about 10 per cent produced flower stalks in 1939, and about 5 per cent in 1940. Although the levulose content did not appear to be affected by this, it is undesirable from the

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<sup>2</sup> Research Associate Professor of Vegetable Crops, and Research Associate Professor and Research Professor of Plant Chemistry, respectively.

agronomic standpoint, since the stems are tough and woody at harvest, which makes them more difficult to "top."

Dahlia varieties were not consistent. In some cases tuberous roots in the peat soil produced more sugar than the same variety in loam, and in other cases the reverse was true. Many of the dahlia varieties produced such small or so few tubers that the yield of levulose per acre was low. Jane Cowle, Yellow Duke, Dwarf Unwin, and Dwarf Coltness were the only varieties producing a satisfactory yield of tubers. The Unwin and Coltness Hybrids have short dwarf plants so that they can be planted close together. About 15 tons per acre of tubers of these varieties were secured, but in both years the percentage of levulose was too low.

No accurate yields by means of replicated plots were taken in either year, but approximate yields taken from one-tenth acre of each are given below.

TABLE 1  
YIELDS PER ACRE

	1939	1940
	Tons	Tons
<b>Chicory</b>		
Loam .....	10	12
Peat .....	13	15
<b>Artichokes</b>		
Sibley strain—loam .....	12	15
<b>Dahlias</b>		
Jane Cowle—loam .....	10	10
Yellow Duke—loam .....	8	10
Unwin—loam .....	12	12
Coltness—loam .....	12	12
Jane Cowle—peat .....	12	8
Yellow Duke—peat .....	10	10
Unwin—peat .....	15	15
Coltness—peat .....	15	15

From results secured in tests for two years, chicory seems to be easier and cheaper to produce from the agronomic standpoint. Of the varieties of chicory tested, Fredonia and Silicion Half-Long are better than Witloof or Magdeburg. Yields of chicory per acre compare favorably with sugar beets, and the levulose content is consistently higher, especially when grown in peat, than that of dahlias or artichokes in either loam or peat.

Advantages and disadvantages of each encountered in Iowa as an agricultural crop and source of levulose are given below.

#### ARTICHOKEs

*Advantages as a source of levulose.*

- a. Large yields of tubers per acre are secured.
- b. Levulose content is high in some varieties.
- c. The crop is adapted to many types of soils.

*Disadvantages.*

- a. Difficult to harvest because tubers of most varieties are produced on long stolons, a considerable distance from the crown or stem of the plant. When digging the crowns, many of the tubers are left in the ground on both sides of the furrow slice, necessitating at least three plowings to each row.
- b. Tops of the plants are difficult to handle at harvest. Tops can be cut with a corn binder, but this leaves an immense amount of material which must be removed from the field before digging can commence.
- c. Tubers left in the ground at harvest produce volunteer plants the following season which necessitate frequent cultivations to destroy them. If shoots from the tuber are destroyed, new shoots will appear above ground several times.
- d. The stolons do not break readily from the crown of the plant, which necessitates hand pulling to remove the tubers.
- e. Storage conditions for tubers are exacting. Storage at a temperature near the freezing point is the only successful method of keeping a large percentage of the tubers free from rot and shriveling for any length of time after they are dug. The poor storage quality of the tubers has an important bearing on any plan to use them for industrial purposes or from an agricultural standpoint where tubers for propagation the following season are necessary.

The Jerusalem artichoke can be planted with a potato planter. One-ounce seed pieces with one or two eyes are sufficiently large to produce strong-growing plants. The ground can be harrowed after the weeds start without injury to the young plants. One cultivation may be necessary between rows when the plants are 6 to 12 inches high. The plants grow so large and dense that all weeds are eliminated by shade after that. Harvesting is costly because "tops" must be removed from the field. A potato digger will not shake the tubers from the crown of the plant.

**DAHLIAS***Advantages as a source of levulose.*

- a. Fair yields of tuberous roots can be secured per acre.
- b. The volume of the tops of the plants to be removed is not as serious as with artichokes.
- c. All tuberous roots can be lifted from the soil in one furrow slice.
- d. If tuberous roots are missed or left in the ground, volunteer plants do not appear the following season in northern United States.

*Disadvantages.*

- a. It is not a good Corn Belt crop since yields are drastically reduced even by short periods of heat or drouth. In other words, it is too sensitive to adverse weather conditions.

- b. Yields of levulose are too low under Iowa conditions to use it as a source of levulose commercially.
- c. The volume of tuberous roots to carry over in storage for propagation the next year is too large and storage conditions necessary for the material are too exacting for average farm conditions if dahlias were grown as an agricultural crop on a large field scale.

Dahlia tubers cannot be planted with a potato planter because of the large size of many tubers. However, they can be dug with a potato digger. Tubers saved for propagation would have to be stored, attached to the stem. The tubers shrivel quickly when exposed to the air. They will not store without break-down near the freezing point. At 40°F. or 45°F., wrapping each clump in paper is the best method to prevent shriveling. This would not be practical on a large scale. Preliminary storage investigations in the winter of 1939-40 of propagating stock showed that dahlia tuber clumps stored in dry sand at 40°F. did not shrivel. This would necessitate a large amount of storage space for propagation the following year. Tubers wrapped in glass wool shriveled so this would not be a satisfactory storage medium.

#### CHICORY

##### *Advantages as a source of levulose.*

- a. Easily propagated from seed.
- b. Yields of tubers high.
- c. Levulose content fairly high.
- d. Roots easily harvested.

##### *Disadvantages.*

- a. Hand labor, such as weeding and preparation of seed bed, is greater than with artichokes, dahlias, or sugar beets.
- b. Roots are smaller than sugar beets, but allow closer spacing, so that yields equal to sugar beets can be secured; however, this increases the cost of topping over that of sugar beets because a larger number of roots per acre must be handled.
- c. Harvesting of all roots in the field is necessary, thus eliminating volunteer plants the following season. The plant is a biennial, and will grow again the following year unless harvesting is complete.

Chicory is propagated from seed, which eliminates the problem of storage of large quantities of propagating material as with the artichoke and dahlia. Weeding and "topping" the roots at harvest would be a little more costly than with sugar beets. Harvesting costs would be far less than with artichokes.

#### CHEMICAL PHASE

##### ANALYTICAL PROCEDURE AND RESULTS

Several of the tubers of different sizes were selected to make a representative sample. They were washed with a brush to remove adhering soil. The washed tubers were then dried with paper towels to remove all the excess moisture. Care must be taken during washing and drying not to remove the skin of the tubers.

The tubers were cut in half and shredded by means of an ordinary household corrugated cabbage shredder. About one-half pound of this wet material was required for analysis. The shreds were spread in a thin layer on a coarse screen and placed in a warm-air dryer. Air at about 50-55°C. was blown over the material until it was dried and crisp on cooling. This process took about 24 to 48 hours. The air-dried material was ground in a Wiley mill to about 40 mesh and stored in Mason jars.

Moisture determinations were made on the air-dried material, and total moisture was determined as follows: Small thin sections were cut out of several different-sized tubers and weighed. The weighed material was dried in a vacuum oven to constant weight.

The survey reported herein was made in 1939 and 1940 with the idea of ascertaining whether these plants contained sufficient levulose to be used as a commercial source.

*Levulose Determination:* The procedure followed was that used by Norman, Wilsie, and Gaessler.<sup>3</sup>

*Glucose Determination:* Total reducing value was determined on an aliquot from the same solution prepared for the levulose determination. This aliquot was taken immediately preceding the sodium hypoiodate oxidation of the glucose sugars. The value obtained in this manner by the Shaffer and Somogyi method<sup>4</sup> and expressed as percentage of total reducing sugars, minus the percentage of levulose, gave the percentage of glucose.

*Moisture and Total Nitrogen:* Moisture of the air-dried sample and total nitrogen were determined by the Official Methods.<sup>5</sup>

Results of analyses are given in Tables 1 and 2.

In 1939, analyses were made on eighteen dahlia, nine artichoke, and eight chicory samples. On a wet basis the levulose content varied from 5.09 per cent to 12.09 per cent for the dahlia samples; from 3.34 per cent to 17.14 per cent for the chicory samples, and from 3.70 per cent to 6.55 per cent for the artichoke samples. The glucose-levulose ratio varied from 0.08 to 0.30 for the dahlia samples; from 0.14 to 0.33 for the chicory samples, and from 0.20 to 0.42 for the artichoke samples. The higher values for chicory were all from samples grown on peat soils.

In 1940, analyses were made on sixteen dahlia, nine artichoke and six chicory samples. On a wet basis the levulose content varied from 2.95 per cent to 12.32 per cent for the dahlia samples; from 13.00 to 18.06 per cent for the chicory samples, and 8.40 to 17.64 per cent for the artichoke samples. The glucose-levulose ratio varied from 0.00 to 0.16 for the dahlia samples; from 0.08 to 0.14 for the chicory samples, and from 0.10 to 0.37 for the artichoke samples. The higher values for the chicory again were all from samples grown on peat soils.

<sup>3</sup> Norman, A. G., C. P. Wilsie, and W. G. Gaessler. The fructosan content of some grasses adapted to Iowa. Iowa State College Jour. of Sci. 15: 301-305. 1941.

<sup>4</sup> Shaffer, Phillip A., and Michael Somogyi. Copper-Iodometric reagents for sugar determination. Jour. Biological Chem. 45: 695-713. 1933.

<sup>5</sup> Association of Official Agricultural Chemists. Methods of analysis. Pp. 227-84. Washington, D. C., 1930.

TABLE 2  
LEVULOSE IN ARTICHOKE, CHICORY, AND DAHLIA GROWN IN 1939

IDENTIFICATION	PCTG. TOTAL MOISTURE	LEVULOSE		GLUCOSE		NITROGEN		GLUCOSE- LEVULOSE RATIO
		Oven	Wet	Oven	Wet	Oven	Wet	
<i>peat</i> .....								
<i>Artichokes*</i>								
Ames seedling ..	74.01	22.54	5.86	5.41	1.41	2.47	.645	.241
Oregon strain ..	80.01	26.63	5.33	6.37	1.27	1.96	.392	.239
Purple Girasole ..	76.92	26.03	6.01	5.12	1.18	1.68	.388	.197
Sibley strain ..	81.91	20.43	3.70	8.51	1.54	2.47	.447	.416
Seedling No. 3 ..	79.67	22.35	4.54	6.79	1.38	2.45	.498	.304
U.S.D.A. 27081 ..	81.74	21.02	3.84	6.94	1.27	2.29	.418	.329
Seedling No. 7 ..	75.90	20.75	5.01	7.86	1.98	2.29	.552	.378
Seedling No. 8 ..	76.92	22.03	5.09	7.08	1.64	2.34	.542	.321
Seedling No. 13 ..	75.32	26.50	6.55	5.22	1.29	2.23	.550	.197
<i>Chicory</i> .....								
Fredonia—Ames ..	78.24	25.75	5.60	5.09	1.11	1.16	.253	.198
Fredonia—peat ..	73.68	64.80	17.04	9.66	2.54	.95	.250	.149
<i>Magdeburg</i> .....								
Ames .....	76.33	24.10	5.70	4.70	1.12	1.45	.315	.196
Magdeburg—peat ..	68.37	54.28	17.14	11.87	3.75	1.23	.388	.219
<i>Magdeburg</i> .....								
seeded—Ames ..	73.39	22.80	6.07	7.58	2.02	1.29	.339	.332
Silicion—Ames ..	77.53	26.15	5.89	3.77	.84	1.25	.278	.143
Silicion—peat ..								
Witloof—Ames ..	79.82	21.45	4.34	5.59	1.13	1.23	.327	.261
Witloof—peat ..	68.84	48.75	15.23	13.29	4.12	1.37	.427	.272
<i>Dahlias</i> .....								
<i>Gertrude</i> .....								
Britton—								
Ames .....	85.68	43.10	6.18	6.94	.99	3.05	.431	.161
<i>Jane Cowle</i> .....								
Ames .....	84.91	52.78	7.98	7.37	1.11	1.72	.258	.140
<i>Jane Cowle</i> .....								
peat .....	80.89	54.40	10.42	12.69	2.42	1.45	.277	.233
Coltness—Ames ..	87.06	56.68	7.33	8.29	1.05	2.02	.262	.146
Coltness—peat ..								
Decorative—Ames ..	85.79	47.30	6.88	7.64	1.09	2.19	.299	.161
Golden Eclipse—								
Ames .....	83.02	46.70	7.94	9.35	1.60	2.21	.375	.200
Nathan Hale—								
Ames .....	80.76	48.55	9.36	14.80	2.90	1.82	.351	.305
Jean Kerr—Ames ..	86.68	45.60	6.08	6.43	.86	2.06	.275	.141
Lake—Ames .....								
Jim Moore—Ames ..	86.49	62.30	8.38	9.36	1.27	2.78	.376	.150
<i>Mixed tubers</i> .....								
Ames .....								
Pompon—Ames ..	80.12	55.23	10.98	4.76	.95	2.82	.522	.086
Seedling No. 1—								
Ames .....	85.42	59.30	8.63	7.39	1.08	1.56	.228	.125
Seedling No. 2—								
Ames .....	83.37	58.30	9.71	7.63	1.27	2.16	.359	.131
Seedling No. 3—								
Ames .....	79.55	58.85	12.09	6.97	1.40	1.94	.398	.115
Unwin—Ames ..	81.34	54.10	10.09	7.47	1.40	1.77	.331	.138
Unwin—peat ..	84.10	48.55	7.72	11.92	1.89	2.24	.356	.246
Mrs. Warner—								
Ames .....	85.35	34.75	5.09	5.40	.79	2.54	.372	.156
Mrs. Warner—peat ..	84.19	43.53	6.87	7.21	1.14	2.14	.398	.165
Yellow Duke—								
Ames .....	84.81	57.00	8.68	7.52	1.14	2.20	.334	.132
Yellow Duke—								
peat .....								

\* All artichoke varieties were grown at Ames.

TABLE 3  
LEVULOSE IN ARTICHOKE, CHICORY, AND DAHLIA GROWN IN 1940

IDENTIFICATION	PCTG. TOTAL MOIS- TURE	LEVULOSE		GLUCOSE		NITROGEN		GLUCOSE- LEVULOSE RATIO
		Oven	Wet	Oven	Wet	Oven	Wet	
<b>Artichokes*</b>								
Ames seedling .....	68.50	56.04	17.84	6.08	1.91	2.110	.665	.108
Oregon strain .....	77.40	52.90	11.96	10.90	2.46	1.954	.441	.257
Purple Girasole .....	76.77	46.17	10.74	7.76	1.80	2.010	.467	.168
Sibley strain .....	80.07	52.32	10.42	19.35	3.86	2.339	.466	.370
Seedling No. 3 .....	80.08	42.16	8.40	12.05	2.40	2.178	.434	.286
U.S.D.A. 27081 .....	77.76	50.69	11.27	13.85	3.06	2.180	.485	.272
Seedling No. 7 .....	77.58	50.51	11.32	14.70	3.30	2.279	.515	.292
Seedling No. 8 .....	78.25	50.74	11.04	14.46	3.15	2.095	.456	.285
Seedling No. 13.....	78.03	46.88	10.32	15.76	3.47	2.358	.518	.336
<b>Chicory</b>								
Fredonia—Ames .....	77.59	61.57	13.80	7.32	1.64	1.000	.224	.119
Fredonia—peat .....	74.43	67.29	17.20	5.36	1.37	1.031	.262	.080
Magdeburg—Ames .....	76.26	63.00	14.95	6.29	1.49	1.265	.304	.100
Magdeburg—peat .....	73.80	68.92	18.06	5.70	1.49	1.218	.319	.083
Ames .....	.....	.....	.....	.....	.....	.....	.....	.....
Silicion—Ames .....	78.58	60.72	13.00	8.71	1.86	1.246	.267	.143
Silicion—peat .....	74.54	64.28	16.36	7.40	1.88	2.592	.659	.115
Witloof—Ames .....	.....	.....	.....	.....	.....	.....	.....	.....
Witloof—peat .....	.....	.....	.....	.....	.....	.....	.....	.....
<b>Dahlia</b>								
Gertrude Britton—Ames	83.80	57.28	9.28	3.35	.54	2.718	.441	.058
Jane Cowle—Ames .....	81.98	67.72	12.32	7.19	1.29	1.022	.184	.105
Jane Cowle—peat .....	87.02	52.57	6.83	6.37	.82	1.838	.239	.120
Coltness—Ames .....	85.91	62.02	8.74	.16	.02	2.201	.310	.000
Coltness—peat .....	86.00	59.74	8.36	5.53	.77	2.212	.310	.092
Decorative—Ames .....	.....	.....	.....	.....	.....	.....	.....	.....
Golden Eclipse—Ames ..	85.66	59.94	7.54	4.47	.64	2.120	.304	.084
Nathan Hale—Ames .....	86.66	51.67	6.70	5.13	.68	2.188	.292	.102
Jean Kerr—Ames .....	85.90	51.59	7.28	6.46	.91	2.354	.332	.125
Lake—Ames .....	90.99	32.82	2.95	4.37	.39	2.820	.254	.132
Jim Moore—Ames .....	88.57	56.41	6.45	6.12	.70	2.065	.236	.109
Mixed Tubers—Ames ..	83.36	64.30	10.69	5.47	.91	1.876	.312	.085
Pompon—Ames .....	.....	.....	.....	.....	.....	.....	.....	.....
Seedling No. 1—	.....	.....	.....	.....	.....	.....	.....	.....
Ames .....	.....	.....	.....	.....	.....	.....	.....	.....
Seedling No. 2—	.....	.....	.....	.....	.....	.....	.....	.....
Ames .....	.....	.....	.....	.....	.....	.....	.....	.....
Seedling No. 3—	.....	.....	.....	.....	.....	.....	.....	.....
Ames .....	.....	.....	.....	.....	.....	.....	.....	.....
Unwin—Ames .....	84.77	57.84	8.81	9.67	1.47	1.901	.289	.167
Unwin—peat .....	87.25	58.68	7.49	5.54	.70	2.049	.261	.093
Mrs. Warner—Ames .....	86.32	49.37	6.76	4.40	.60	2.376	.325	.089
Mrs. Warner—peat .....	.....	.....	.....	.....	.....	.....	.....	.....
Yellow Duke—Ames ..	86.86	57.78	7.60	1.92	.25	2.658	.349	.033
Yellow Duke—peat .....	85.49	53.93	7.82	6.61	.96	2.132	.309	.123

\* All artichoke varieties were grown at Ames.



SOME MAJOR FACTORS AFFECTING THE USE OF TWO  
INVENTORY METHODS APPLICABLE TO THE WESTERN  
FOX SQUIRREL, SCIURUS NIGER RUFIVENTER  
(GEOFFROY)<sup>1,2</sup>

ELLIS A. HICKS

From the Department of Zoology and Entomology, Iowa State College

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The fox squirrel, *Sciurus niger rufiventer* (Geoffroy), is an important game animal found throughout Iowa, active the year around. In the proper management of a species there must be some reliable method by which knowledge of population fluctuations can be obtained.

Goodrum (3) described several census methods used to determine squirrel populations in Texas. Chapman (2) and Baumgartner (1) outlined several census techniques applied to squirrel studies in Ohio.

In selecting the area on which observations were to be made, it was thought advisable to choose a tract on which there was very little hunting. Although the influence of this factor was confined to open season during the fall months, elimination of this variable for the most part led to simpler interpretation of data. The area of investigation consisted of 250 acres along Squaw Creek in Sections 29, 32, and 33, Franklin Township, Story County. Most of the area was subject to grazing by cattle, and the few ungrazed tracts did not have sufficient underbrush and second growth to interfere with enumeration. There were 100 acres of flood plain, chiefly a weedy pasture with American elm (*Ulmus americana*), silver maple (*Acer saccharinum*), and black walnut (*Juglans nigra*) as the dominant trees. The rough terrain consisted of 150 acres of wooded ridges and gullies. This tract supported chiefly an oak-hickory stand with a mixture of other trees of lesser importance. In this investigation two methods were used. The first can be termed simply a "linear" count which involves use of a definite route of known width and length with tabulation of all individuals seen. The several routes used varied from  $\frac{1}{4}$  to 2 miles in length and varied in width depending upon seasonal factors, chiefly degree of defoliation. Selection of routes was made to include a wide variety of vegetation and of topographic contour.

The second method is known popularly as the "spot" count. For this method two adjacent tracts of approximately 20 acres each were selected. Both were comparable as to vegetative types and topography. Each observation lasted 30 minutes. Four observations each half day were

<sup>1</sup>Abridged form of Master of Science Thesis, Library, Iowa State College. The research was under Dr. Geo. O. Hendrickson, Iowa State College, and Thos. G. Scott, U. S. Fish and Wildlife Service.

<sup>2</sup>Journal Paper No. J-887 of the Iowa Agricultural Experiment Station, Ames, Iowa. Project No. 599. Fish and Wildlife Service (U. S. Department of the Interior), Iowa State College, Iowa State Conservation Commission, and the American Wildlife Institute cooperating.

TABLE I  
RELATION BETWEEN TEMPERATURE AND SQUIRREL COUNTS

Temp. in Degrees F.	SPRING		SUMMER		FALL		WINTER		SEASONS COMBINED		
	Squir- rels Seen	Hours Obs. Time	Squir- rels an Hr. Seen	Hours Obs. Time							
1- 10 .....	.....	.....	.....	.....	.....	0	2	0	0	2	0
11- 20 .....	.....	.....	.....	.....	.....	13	8	1.63	13	8	1.62
21- 30 .....	.....	.....	.....	.....	18	4.5	4.00	8	14.5	0.55	26
31- 40 .....	10	10	1.00	.....	28	9	3.11	5	4	1.25	43
41- 50 .....	20	10	2.00	.....	16	11	1.46	2	2	1.00	38
51- 60 .....	9	8	1.13	1	2	0.50	5	1.5	3.33	.....	15
61- 70 .....	8	8	1.00	17	12	1.42	7	12	0.58	.....	32
71- 80 .....	36	27	1.33	3	8	0.38	16	6	2.66	.....	55
81- 90 .....	12	14.5	0.83	7	10.5	0.67	.....	.....	.....	19	25
91-100 .....	0	2	0	6	5	1.20	.....	.....	.....	6	7
Totals .....	95	79.5	.....	34	37.5	.....	90	44	.....	28	30.5
										247	191.5

made, with two observations occurring on one tract and two on the other. As far as possible, random selection of count spots was made at all times.

Application of the census methods was directly affected at all times by several variables. There were definite preferences for certain vegetative types, especially in connection with food. The oak-hickory stands furnished a large portion of the food; hence, during feeding hours more squirrels were found in such areas than in stands of trees which did not furnish food. Preference for certain trees for construction of den nests and leaf nests also affected the amount of activity in various tree stands. Possibly topographic differences such as gully, ridge, or flood plain also had an effect on distribution of activity. Attempts were made to evaluate the effects of these factors. Undoubtedly, temperature was an exceedingly important factor in determining the degree and possibly, to a lesser extent, the time of activity. In midsummer heat, strenuous and continued exertion was not nearly so common as during moderate temperatures. Extreme cold also had the effect of decreasing activity. In conjunction with temperature, another important factor was time of day at which observations were made. Possession of rather definite feeding hours, indicated by many of the animals, tended to group movements into a peak or peaks for the day. Lengthening and shortening daylight hours in accordance with the seasons controlled duration of activity. Other factors attributable to seasons were mating, rearing of young, and preparing for winter. Here entered the influence of nest building and storing foods. Several meteorological factors had direct effects. Precipitation, either rain, sleet or snow, and high humidity in the form of fog or mist decreased the amount of movement noticeably, and high winds tended to decrease movement. As can be readily seen, the chief difficulty in analyzing each observation lies in the fact that almost every time there were several factors acting simultaneously.

In an attempt to explain the relationship of temperature to animal activity, the data are presented in Table 1. An examination of the table indicates that the relationship of squirrels seen and observation periods is affected by factors other than temperature. Ratio values for fall, with an average variation of 1.85, are far more erratic than those for any other season. It is possible that these extreme variations are caused in part by such factors as nest building, gathering food supplies, and other preparations for winter. Spring, summer, and winter variations of ratio values, 0.57, 0.70, and 0.68, respectively, are quite close together. Even with the latter three seasons there is so much variation among ratio values that no definite conclusions can be drawn concerning the effects of temperature alone upon squirrel activity.

A consideration of ratio values for the various temperature brackets of all seasons combined shows some interesting trends. The average variation of ratio values for all seasons combined is 0.33. The ratio values rise rather abruptly to a maximum at the 31-40 degree temperature brackets and gradually decline with increase in temperature.

Although with some animals we often associate sunny weather with more pronounced activity, there is a certain fairly constant amount of activity regardless of the degree of clearness. Hunger is an impelling force to be satisfied, and neither sunshine nor clouds vary the amount of time spent in foraging as greatly as other activities of a minor nature. In regard to these other types of behavior, which may be included in the terms exploration, play, and loafing, cloudiness may exercise a direct influence.

An examination of Table 2 shows that the ratio of squirrels seen hourly on cloudy days is lowest of the three tabulated ratios. Partly cloudy days yield a sizeable ratio increase, but the ratio increase from

TABLE 2  
RELATION BETWEEN DEGREE OF CLOUDINESS, ANIMAL ACTIVITY AND OBSERVATION TIME

	No. of Observation Periods	No. of Squirrels Seen	No. of Hours of Observation	No. of Squirrels Seen an Hour
Cloudy .....	100 (26.10%)	47 (19.05%)	50	0.94
Partly cloudy .....	78 (20.38%)	47 (19.05%)	39	1.21
Clear .....	205 (53.52%)	153 (61.90%)	102.5	1.49
Totals .....	383	247	191.5	.....

partly cloudy to clear is almost identical with that from cloudy to partly cloudy. From these figures it is apparent that complete cloudiness does have an important effect upon the amount of squirrel activity. Rain, snow, mist, and other forms of precipitation would be expected to decrease activity. The figures for partial cloudiness indicate that intermittent sunshine tends to increase squirrel activity. The maximum amount of activity for unit of time is indicated on clear days. There has been mentioned previously the likelihood that degree of cloudiness does not affect feeding as greatly as other factors. However, observations indicate that such activities as playing and loafing are definitely affected by the degree of cloudiness. Consequently, cloudiness can be considered an important influence on squirrel activity as a whole.

In attempting to account for the variations of ratio values given in Table 3, there are several factors which undoubtedly exerted some degree of influence on monthly squirrel activity.

It will be noticed that the January ratio of squirrels seen for observation time is 0.67, a relatively low ratio. With February the ratio increases to 1.00. Corresponding ratios for succeeding months fluctuate slightly below and above 1.00 until July. From February to June inclusive, moderation of temperatures, increased variety of food supply, and brood care help to increase the amount of animal activity over that of January when cold snaps often serve as deterrents to squirrel movement. July witnesses a noticeable decrease which is yet discernible in August. Undoubtedly the major cause for this is midsummer heat and accompany-

TABLE 3

RELATION BETWEEN MONTH OF YEAR, TOTAL NUMBER OF OBSERVATION PERIODS, TOTAL NUMBER OF SQUIRRELS SEEN, TOTAL NUMBER OF ACRES OBSERVED, RATIO OF SQUIRRELS SEEN TO HOURS OF OBSERVATION TIME, AND RATIO OF SQUIRRELS SEEN TO ACREAGE OBSERVED

	Number of 30-Minute Observation Periods	Number of Squirrels	Acres Observed	Squirrels an Hour	Squirrels an Acre
<b>Winter</b>					
Dec. 21-31 .....	3	5	109	3.33	0.05
Jan. .....	18	6	114	0.67	0.05
Feb. .....	20	10	125	1.00	0.08
Mar. 1-20 .....	20	7	125	0.70	0.06
Totals .....	61	28	473	0.92	0.06
<b>Spring</b>					
Mar. 21-31 .....	12	8	75	1.33	0.11
Apr. .....	42	25	232	1.19	0.11
May .....	71	37	122	1.04	0.30
June 1-20 .....	22	12	26	1.09	0.46
Totals .....	147	82	455	1.12	0.18
<b>Summer</b>					
June 21-30 .....	24	16	42	1.33	0.38
July .....	8	1	12	0.25	0.08
Aug. .....	23	8	82	0.69	0.10
Sept. 1-20 .....	32	22	112	1.38	0.20
Totals .....	87	47	248	1.08	0.19
<b>Fall</b>					
Sept. 21-30 .....	12	6	18	1.00	0.33
Oct. .....	42	39	136	1.86	0.29
Nov. .....	30	36	537	2.40	0.07
Dec. 1-20 .....	4	9	55	4.50	0.16
Totals .....	88	90	746	2.04	0.12

ing animal lethargy. With the approach of autumn, however, a conspicuous increase in animal activity is shown. The peak is reached in December, with a ratio value of 4.50. From September to December a major portion of the squirrels' time is devoted to foraging and laying in a supply of winter foods. Also at this time there is construction of twig-and-leaf nests. Addition of the year's brood with an increased squirrel population is another factor to consider as effecting the greater ratio values.

An examination of the relationship between months and ratio of number of squirrels to acres observed shows that the months fall into two distinct categories. During January, February, March, April, November, and December, trees and shrubs are defoliated. This greatly increases range of vision with consequent increase of acreage values. This tends to keep the ratio values small. From May to October, inclusive, however, foliage delimits vision and the relatively small acreages surveyed during each observation period increase the ratio values. In the case of July and August, the decreased squirrel activity lessens noticeably the squirrel-acreage ratios in spite of the comparatively smaller areas under observation.

In Table 4 is shown the influence which time of day has on squirrel movement for both methods of inventory. A limited number of observations were made prior to 8:00 A.M., and after 5:00 P.M. At the morning observations, activity was noticed to increase gradually to the forenoon maximum. In late afternoon there was a continuation of the decrease in activity from the early afternoon peak. The high ratio values for the forenoon hours 8:00-11:00 contrast greatly with the low ratio values for the remainder of the day. Beginning at 11:00 A.M. there is a sharp

TABLE 4  
RELATION OF TIME OF DAY, NUMBER OF 30-MINUTE OBSERVATION PERIODS, NUMBER OF SQUIRRELS, AND RATIO OF SQUIRRELS TO HOURS

TIME OF DAY	LINEAR METHOD			SPOT METHOD		
	No 30-Min Observation Periods	No of Squirrels	Squirrels an Hour	No 30-Min Observation Periods	No of Squirrels	Squirrels an Hour
8- 9	10	9	1.80	33	34	2.06
9-10	16	32	4.00	63	58	1.84
10-11	13	15	2.31	41	33	1.61
11-12	2	1	1.00	5	1	0.40
1- 2	12	9	1.50	22	10	0.91
2- 3	22	13	1.18	62	8	0.26
3- 4	17	8	0.94	42	7	0.33
4- 5	13	7	1.08	6	0	0.00

drop in the ratio of squirrels seen to hours of observation. A slight increase is evident for the hour 1:00 P.M.-2:00 P.M., but after this time the values are relatively small. The linear method indicates the peak of activity for the forenoon as well as for the whole day occurs at 9:00 A.M.-10:00 A.M. The afternoon peak of activity is reached between 1:00 P.M. and 2:00 P.M. With the spot method of inventory again are emphasized the high ratio values for the forenoon hours from 8:00-11:00. However, in use of the spot method the activity peak for the day occurs from

8:00 A.M.-9:00 A.M. instead of from 9:00 A.M.-10:00 A.M. as in the case of the linear method. Afternoon peak of activity for the spot method is included between 1:00 P.M.-2:00 P.M. as is true with linear count. Both methods emphasize the fact that there is much greater activity in the forenoon than in the afternoon.

#### SUMMARY

1. To learn something of the factors affecting activity of the fox squirrel, observations were made on a 250-acre timbered area in Story County over a period of 1.5 years.
2. Two inventory methods known as linear count and spot count were used in the investigation.
3. Use of these methods was greatly influenced by a large number of factors, namely, temperature, degree of cloudiness, topography, vegetative distribution and association, precipitation, season of year, hour of daily observation.
4. Yearly observations indicate that the temperature bracket 31° F. to 50° F. was most conducive to squirrel activity.
5. Much more activity occurred on clear days than on cloudy days.
6. Seasonal division of data indicated that the peak of activity occurred in fall. Activity during each of spring and summer was about half that of fall, while winter movement was slightly less than that in either spring or summer.
7. The two methods of count substantiated each other in indicating the time of day during which squirrel activity was most pronounced. The peak of activity occurred from 8:00 A.M.-10:00 A.M. with a minor surge from 1:00 A.M.-2:00 P.M.

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## NUCLEAR APPARATUS AND SEXUAL MECHANISM IN A MICROCOCCUS

CARL C. LINDEGREN

*From the Department of Bacteriology, University of Southern California*

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### THE GENETICAL AND MICROSCOPIC METHODS OF DEMONSTRATING THE EXISTENCE OF A NUCLEUS

Because the nucleus is the fundamental biological structure, the visual demonstration of a bacterial nucleus is of basic importance from the standpoint of research which deals with the biology of bacteria. This fact has been brought into sharp focus by those studies in bacterial variation which are concerned primarily with the mechanism rather than the description of variation. There are two methods by which nuclei of higher forms have been shown to exist. These are the genetical and the microscopical methods.

The genetical method uses data accumulated by studying the hereditary transmission (or nontransmission) of variation in fungi and other plants and animals. These data prove that a definite structure exists which carries the hereditary factors. This proof is based on purely mathematical grounds and is completely independent of microscopical demonstrations. These numerical data accumulated by genetical experiments show that particles, called genes, are present in plants and animals, arranged in gene strings and that each string splits longitudinally at each cell division. The genes are the differentiators (rather than determiners) which give the different variants ("dissociants") of plants and animals their different characters.

The microscopical demonstration of the nucleus shows that particles called chromomeres (generally supposed to correspond to genes) are arranged linearly in structures called chromosomes (corresponding to the geneticist's gene strings). The chromosomes multiply by longitudinal division, and an equal half of each chromosome passes to each daughter nucleus. This circumstance identified the visible nucleus as the carrier of the hereditary factors and provided a physical structure which satisfied the geneticist's concept of the gene string. In the absence of such a visual demonstration the formal genetical concept of the gene string would have been applied without prejudice to all living forms including the bacteria. Geneticists familiar with the formal concept of the gene string and the function of the genes in transmitting hereditary variations feel safe in the generalization that such a structure is a universal characteristic of all living organisms, whether or not it may be morphologically demonstrable. The stability of the hereditary characters of bacteria is evidence for the view that a gene string, essentially

similar to that found in other living organisms, is probably the principal governing agent in the heredity and variability of bacteria.

The visual demonstration of a nucleus is easiest in forms with large cells and large nuclei, and biologists avoid study of small-celled plants and animals. Liliaceous plants and grasshoppers are preferred solely on the basis of nuclear size. Since size is the most important factor in this visual demonstration, forms as small as bacteria are extremely unfavorable cytological material. The most delicate phase of cytological technique involves the differentiation of the different internal components of the cell. Bacteriological staining, on the other hand, has been developed primarily as a technique of staining the *entire* cell, and bacteriologists have not generally used the standard cytological methods for distinguishing nucleus and cytoplasm.

#### THE PROBLEM OF PROVING THE EXISTENCE OF NUCLEI IN FUNGI

Fungi are often considered to differ from bacteria by the possession of demonstrable nuclei, but in many fungi, it is practically impossible to demonstrate by microscopy that the vegetative nuclei are true nuclei (13). The vegetative nuclei of most fungi are merely very small, densely staining particles. Mitoses of these nuclei with demonstrable chromosomes have been observed, to my knowledge, in only two isolated cases. Dividing vegetative nuclei are visible only as minute dumbbell-shaped particles in which no details of structure can be differentiated. True nuclei can be seen in the fungi immediately before and after the time of sexual fusion. This fact has led to the supposition that the minute, densely staining granules in the vegetative hyphae are true nuclei, because they produce structures in the sexual phase which are identical with the nuclei of higher forms. Actually, the view that the fungi contain nuclei is merely a deduction from the generalization that all living things must contain nuclei. It is only the genetical evidence, which proves the gene strings maintain the genes in regular order throughout the period of vegetative growth, that clearly shows the dumbbell divisions must have been mitotic divisions. In many fungi, a sexual stage has not been discovered and true nuclei cannot be seen. But these imperfect fungi transmit their hereditary characters with a high degree of constancy and produce variants in a manner similar to forms that are known to be nucleate, thus indicating that the hereditary mechanism is essentially similar for all fungi, even in those whose nuclei are so small that conclusions based on a study of the visible structures would be of questionable value. The close morphological relation of this type of fungus with bacteria and the similar degree of stability and variability in transmission of hereditary characters justifies the postulation of the same sort of nuclear mechanism in bacteria, at least until some other and different mechanism can be demonstrated.

#### "DIFFUSE" NUCLEI IN DROSOPHILA AND MAIZE

It is often implied that if nuclei are present in bacteria, one should be able to demonstrate them cytologically in any bacterial cell. This

point of view is an exact parallel to the first objections that were made to the chromosome theory. The chromosome theory assumed that the chromosomes in higher plants and animals maintained their individuality in the resting nuclei although they were not demonstrable by microscopy at this stage. No rigid microscopical proof of the fact that the chromosomes, which reappear at each cell division, have maintained their individuality in the interim has ever been produced, and it is genetical data alone which proves that the gene string has remained intact throughout the life of the organism, whether or not it is encrusted with the chromatin which makes it visible.

Since the connections between the chromomeres are not visible in the resting nucleus of higher forms, no chromosomes (gene-strings) are demonstrable at this time, but the geneticist justifies the assumption that such a nucleus contains gene-strings on the basis of the stability of the hereditary characters. Bacteria are similarly stable, and it is reasonable to assume a similar mechanism in bacteria. The absence of visible connections between the chromomeres in the resting nuclei of higher organisms, like *Drosophila* or *Zea*, makes all stained, resting stage nuclei look as if the chromosomes were "diffuse." Although the "diffuseness" of *Drosophila* chromosomes is no longer considered seriously, the opinion that bacterial nuclei are probably diffuse is often heard. The obvious objection to this view is that a nucleus is a structure whose principal function is to maintain intact the total complement of genes in a definite linear order, and any diffusion of such a structure renders it incapable of performing this special function. Genes free in the cytoplasm and separated from their neighbors would be unable to transmit the hereditary characters with any constancy. If genes were free-living in the bacterial cytoplasm, the frequency of variation would be greater than anyone has hitherto encountered.

Guilliermond (2) has described what he calls "primitive" nuclei in *Rhodothiobacteria* and has confirmed the findings of the Hollandes with respect to similar structures in the anthrax and typhoid bacillus. Because these nuclei divided by simple constriction, without the formation of visible chromosomes or spindle fibers, he considered them to be nuclei of a primitive type. It is clear from the preceding discussion that if there is any connotation of random distribution of the hereditary materials in the word "primitive" as used by Guilliermond, it is not a permissible descriptive term. Belar (1) showed that all the previously reported primitive protozoal nuclei which he and other competent workers reinvestigated with modern fixing and staining techniques, showed true spindles and chromosomes.

#### GENIC VERSUS CYTOPLASMIC HEREDITY

Genetical work on higher forms has revealed that transmissible variation is principally controlled or differentiated by genes or gene-like plasmids. I have studied critically Jollos' (6) experiments on protozoa which he concluded were evidence of cytoplasmic inheritance. In this case gene

mutation probably explains the original variation; the reverersions were probably due to reverse mutations. I have reported similar cases in fungi and bacteria. Hadley (3) has recently again proposed that bacterial variation is cytoplasmic. But our interpretation of bacterial variation must be deductive, based on the generalization developed in the study of higher plants, animals, and fungi, and the prime generalization developed in this work is that the nucleus is the principal agent controlling heredity. This does not mean, however, that all bacterial variation is genic. Environmental conditions produce modifications which disappear when the conditions are altered.

There are also truly cytoplasmic effects. In a recent revolutionary analysis of the genetics of yeasts, Winge and Laustsen (14) have shown that true cytoplasmic inheritance is experimentally demonstrable. This work is free from the errors encountered in Jollos' (6) earlier studies and is, in fact, the most perfect demonstration of a transmissible cytoplasmic effect at present available. These cytoplasmic effects principally involved reduced size and decreased fertility.

In addition, Winge and Laustsen have demonstrated that rough and smooth colony form, cell shape, and the specific capacity to ferment certain sugars are definitely gene-controlled. These are the characters most frequently used by bacteriologists in the study of bacterial variation. This conclusive analysis makes it reasonable to assume that in bacteria, these characters are similarly gene-controlled, or as Bridges would say, "gene-differentiated."

These considerations make it especially interesting to determine if structures can be demonstrated in bacteria which resemble standard chromosomes and nuclei. I have tried to make it clear, however, that one cannot expect such a demonstration to be without gaps, since even in higher plants and animals, no demonstration of the individuality and continuity of chromosomes and nuclei based on microscopy alone is available.

#### SOURCE OF THE CULTURES

The present paper deals with the nuclear apparatus and sexual mechanism of a non-acid-fast diplococcus-tetrad which Mellon, Fisher, and Richardson (12) described as a dissociant of the tubercle bacillus. This organism was obtained by filtering cultures of the tubercle bacillus and then planting the filtrate on agar plates and serially streaking from this first plate on to other agar plates. The cultures appearing on the plates after this procedure contained two dominant forms, a non-acid-fast diplococcus-tetrad and an acid-fast diplococcus-diphtheroid. Both of these cultures when young are apparently pure cultures of diplococci. The tetrads and diphtheroids develop subsequently, hence, diplococcus-tetrad and diplococcus-diphtheroid. In my hands the diphtheroids and tetrads maintained their purity and showed no tendency to transform into each other, but I performed no experiments aimed at transforming them.

The view that these bacteria are cyclo-stages of the tubercle bacillus was developed by Dr. Mellon, and my participation in this work was confined to a cytological and genetical study of the diplococcus-tetrad. The genetical study (a series of three papers in *Zeit. f. Bakt.*, 1935 and 1936) concerned the mutation of normal to G-type colonies. A preliminary paper on the cytology of this organism was published in 1932 (Lindegren and Mellon (7)). The interpretation in the present paper is the result of much longer study and differs from that in the preliminary report. The photographs were made in Dr. Mellon's laboratory in 1933.

In 1940, Miss Jane Cravens, in my laboratory at the University of Southern California, studied the internal structures of a bacterium isolated from the air. This organism was identified as Bergey's *Micrococcus ochraceus* by the various cultural and morphological criteria employed in the Manual of Determinative Bacteriology. Cytological study revealed various morphological forms with internal structures indistinguishable from those shown in the photographs (Figs. 1-28) made several years earlier in Dr. Mellon's laboratory with the organism isolated from the tubercle bacillus filtrates. Other cultures of *Micrococcus ochraceus* showed these forms, which probably indicates that any culture of *M. ochraceus* will reveal similar structures.

These facts have no bearing on whether or not Dr. Mellon's organism is a true cyclo-stage of the tubercle bacillus. This question can only be answered by a repetition of his experiments. It does not seem to me that the fact that *M. ochraceus* is a sufficiently constant and stabilized form to merit status as a species prejudices the question one way or another. There are numerous examples in the fungi where different phases of the same fungus are recognized by authorities as separate true species for many years until finally some investigator integrates these "cyclo-stages" into a single species. One difficulty in integrating *M. ochraceus* into any cycle is the frequency with which it occurs as an air-borne contaminant. Dr. Mellon has transformed the diplococcus-diphtheroid into the R form of the tubercle bacillus but has not studied the diplococcus-tetrad (*M. ochraceus*) with this end in view.

#### STAINING TECHNIQUE

The preparations shown in the photographs were made as follows: A cover-glass was first smeared thinly with Mayer's albumen by putting an extremely small drop of albumen on the cover-glass and then spreading it with a clean, dry finger tip. A needle containing bacteria picked from a colony was then streaked thinly over the albumen. A drop of aceto-carmine was placed on a slide and the cover-glass inverted over it. For a study of the internal structures of bacteria this type of wet mount is essential, for dehydration and mounting in balsam causes so much shrinkage that most of the internal detail is lost. The

wet mount is heated gently and sealed with a mixture of gum mastic and paraffin. Such preparations will last about a month.

Another stain gave comparable results. This was Barrett's haematoxylin mixture (1 part 4% ferric ammonium sulfate, 1 part .5% haematoxylin, 2 parts glacial acetic acid). The acetic acid in both these stains causes considerable swelling in addition to killing and fixing, and this is a marked advantage. The magnification of the photographs in the figures is 3,900.

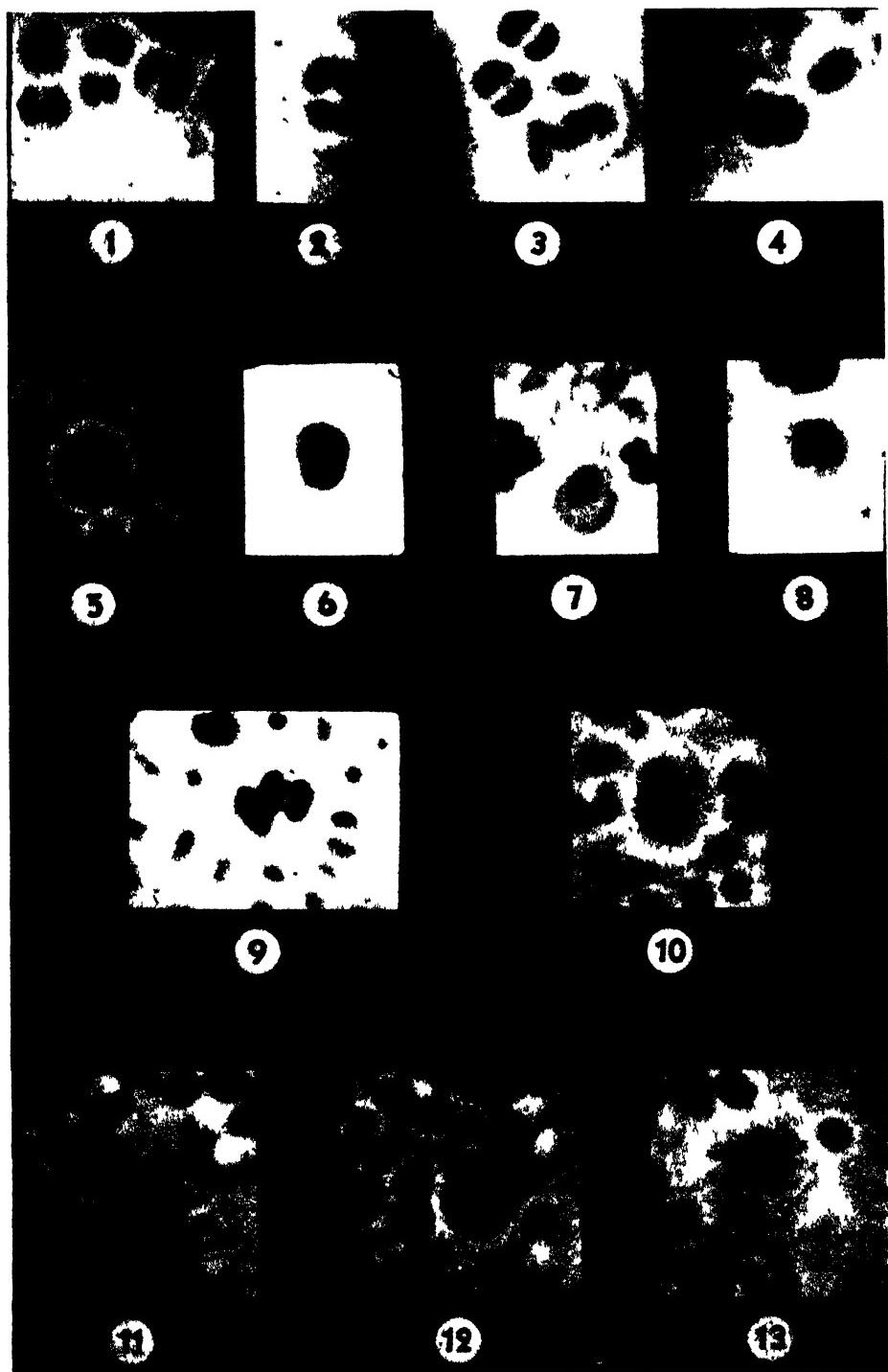
#### ANALYSIS OF INTERNAL STRUCTURES

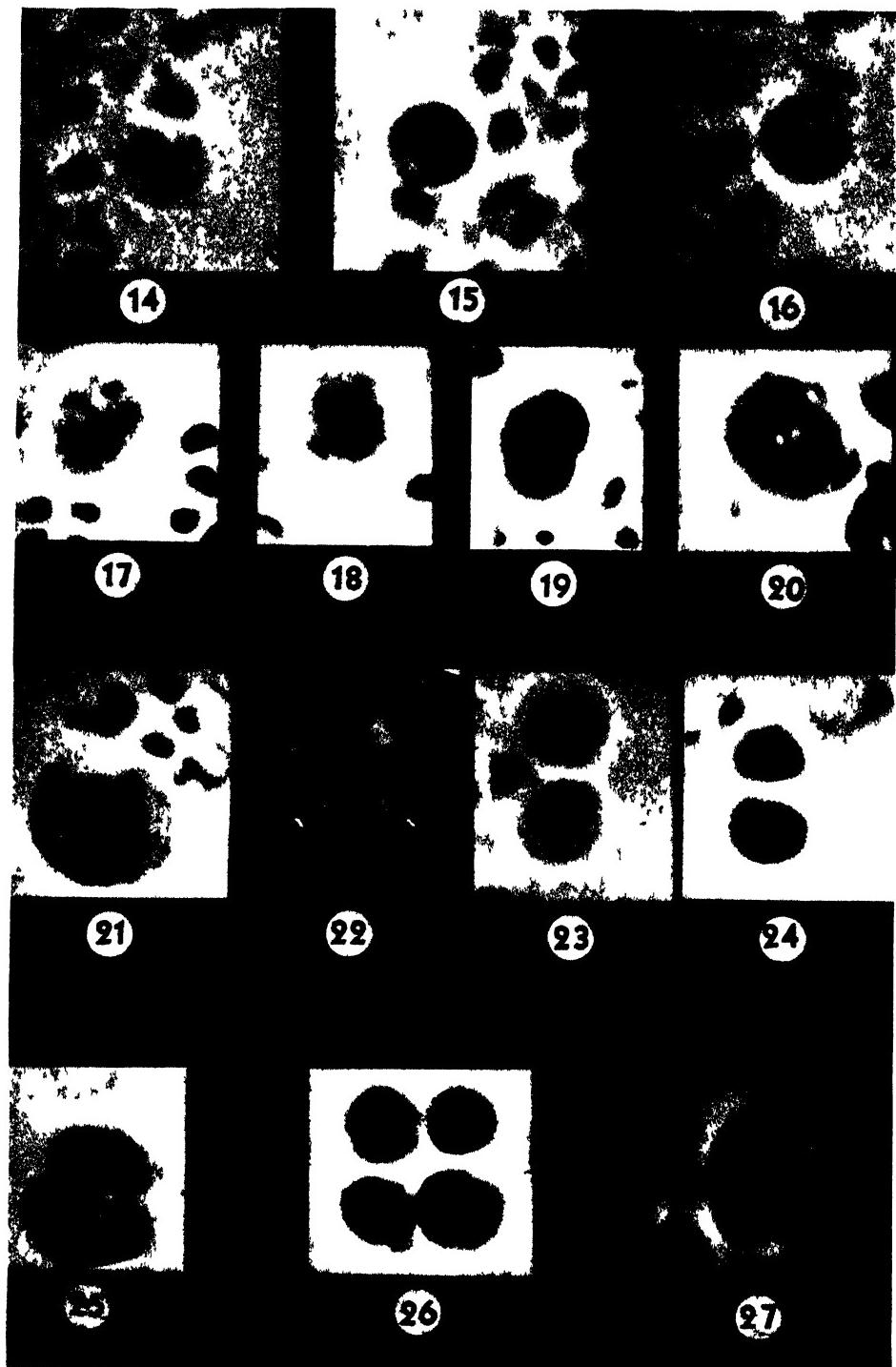
The cultures whose "involution" forms are reported in this paper originated from single cell isolations of the large tetrads found in Dr. Mellon's cultures. These large tetrads (Fig. 27) were observed under the microscope to germinate directly by continued subdivision into small diplococci less than a micron in diameter (Figs. 1, 2, 3, 4, upper left) and produced cultures which at first contained only diplococci. No diphtheroids developed in any cultures. When the cultures were a few weeks old, they contained a few large coccoidal forms in addition to an abundant background of tiny diplococci. Several hundred single cell cultures were made from single diplococci, and in every case large coccoidal forms and macrotetrads arose in the cultures after an initial growth of tiny diplococci had occurred. A critical cytological study of these "involution" forms revealed that they contained structures closely resembling chromosomes and nuclei.

The living diplococci have been observed dividing under the microscope. In these divisions each new diplococcus originates from a single half of a preceding diplococcus. The alternative possibility, namely, that each diplococcus is produced from half of the upper and half of the lower parts of the parent diplococcus is excluded. In Figure 1, diplococci are shown before division. Figure 2 shows a dividing diplococcus. The original plane of separation is horizontal, and the tetrad will split on this plane. The new plane is vertical. In Figure 3 the two new diplococci are swinging apart (Fig. 28, a, b, c). The ordinary vegetative diplococci which predominate in the early stages of growth stain evenly and show no internal structures. They may vary somewhat in size from the small forms in the upper left of Figure 4 to those in Figures 1, 2, and 3.

The first evidence of differentiation in the protoplast is the appearance, in a slightly enlarged diplococcus, of a darkly staining body in each cell (Fig. 4). These bodies resemble fungal nuclei. If they are nuclei, then it is reasonable to suppose that each half of the vegetative diplococci likewise contains a single nucleus which cannot be made visible by staining because of the density of the cytoplasm. This, by the way, is one of the commonest difficulties encountered in cytology of the fungi.

After the appearance of these nuclei in each half of the diplococcus, the intervening wall disappears and the two nuclei become approxi-





mated (Fig. 5). After the nuclei have approximated, a single chromosome begins to unfold from each (Figs. 6, 7, and 8). The fact that a single chromosome arises from each nucleus indicates that each coccus is haploid.

Figure 9 shows the leptotene. The chromosomes have spun out into tortuous spirals. The outline of the cell in this figure is rather faint. The two chromosomes are closely associated but have not yet fused. Figure 10 shows the fused chromosomes at early pachytene. The chromomeres are clearly visible and can be counted. Seven or eight of the chromomeres have fused, and a terminal pair of chromomeres is still unpaired. The entire contents of the cell appear in a single focus.

The next stage is diplotene. Figures 11, 12, and 13 represent upper, middle, and lower focus of a single cell at diplotene. In this case three foci were necessary to make the entire contents of the single cell visible. It is possible to see about double the number of chromomeres that was present at leptotene. Presumably the chromosome has split to prepare

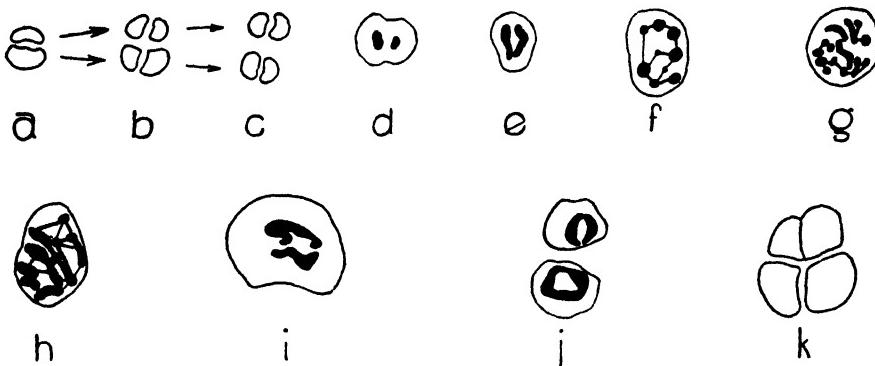


FIG. 28.—Interpretation by line drawings of some of the photographs shown in Figures 1 to 27.

*a* is a drawing showing a single diplococcus shown in *a*, divides to form a small tetrad as shown in *b*, and this tetrad separates to form two diplococci on the original plane of division as shown in *c*.

*d* is a drawing showing the two nuclei in a single swollen diplococcus in which the dividing wall has lost its original distinctness.

*e* is a drawing of Figure 6, showing two chromosomes which have spun out, one from each of the original nuclei shown in *d*.

*f* is a line drawing of Figure 10, showing a single string of seven synapsed chromomeres with the terminal pair of unsynapsed chromomeres.

*g* is a drawing of the single cell shown in three foci in the photographs (Figs. 14, 15, and 16). A larger number of chromomeres is visible with numerous interconnections rather difficult to follow.

*h* is an interpretation of photographs (Figs. 18 and 19) showing a cell almost filled with a large reticulate nucleus carrying a considerable amount of chromatin in a complex net.

In *i* the two chromosomes visible in Figure 21 are shown at the stage just before the first reduction division.

*j* is an interpretation of Figure 24. The first reduction division has been completed, and the chromosomes have formed preparatory for the second reduction division.

*k* is a drawing of the macrotetrad shown in Figure 27.

for the first meiotic division. Figures 14, 15, and 16 similarly represent three foci of another cell at the same stage.

Next in the size sequence is the type of cell shown in Figures 17, 18, and 19. A typical reticulate nucleus occupies the center of the cell. Figure 18 is a light print of Figure 19. In Figure 20, the reticulum has condensed, and in Figure 21 two chromosomes are seen emerging from the nucleus.

Figures 22, 23, and 24 show the first reduction division which produces a large, two-celled form with a nucleus in each cell. In Figure 25 the second reduction division has completed, and a four-celled form results with a single nucleus in each quarter. Figure 26 shows the beginning of the fragmentation of the macrotetrad into small diplococci.

The mature viable macrotetrad is shown in Figure 27. This form is relatively resistant. Single macrotetrads will grow when isolated from a culture two months old. Single diplococci seldom produce cultures when isolated from cultures more than a few days old. Although there is quite a size range in the small viable diplococci, the macrotetrads are distinct structures and are clearly different from both the large coccoidal bodies and the small diplococci.

#### SIGNIFICANCE OF "INVOLUTION" FORMS

The intermediate coccoidal "involution" forms (Figs. 4 to 26) have often been isolated and observed, but they did not change shape or continue to grow during the period of observation; they are nonviable. They may be cells which started to grow into macrotetrads and then degenerated or died. However, their internal structures are reflections of previously existing functional cell inclusions and are valuable as indications of processes occurring in normal cells which might not be capable of differentiation by staining techniques. The best cytological information in the study of *Drosophila* is obtained from the abnormal and monstrous chromosomes of the salivary glands of that organism. For many years these abnormal chromosomes were considered to be extreme artifacts involving almost total degeneration of the cell. Recently, critical study has shown them to be true chromosomes more than a hundred times larger than ordinary chromosomes and therefore much easier to study. Involution forms of bacteria probably afford similar valuable devices for overcoming the difficulties which small size usually imposes upon cytological examination of these forms.

#### THE SEXUAL CYCLE IN *M. OCHRACEUS*

In this critical study of the "involution" forms of a *Micrococcus*, the internal structures are interpreted as nuclei and chromosomes. It appears that these structures are involved in a sexual mechanism. The separate observations of different morphological forms in killed stained preparations were integrated into one scheme by arranging them into a sequence according to size, although this sequence was not observed to occur

under the microscope in living material. No attempt was made to select forms that fitted into a sequence artificially; size relations were the only ones considered. In the smallest forms, the vegetative diplococci, no internal structures were observable. In the next stage the cells were somewhat larger and a stainable nucleus was present in each half of the diplococcus. The wall separating these nuclei subsequently dissolved, and each nucleus spun out to form a single chromosome. These chromosomes paired and then continued to elongate. Cells containing about eight chromomeres, each one presumably a fusion chromomere, were found. If this interpretation is correct, it would indicate that the haploid number of chromomeres (genes?) is about eight. The next largest cells showed about 16 chromomeres, indicating that the string had split. In the succeeding stage the cells contain reticulate nuclei. Eventually each of these nuclei formed two chromosomes. These chromosomes were separated at the first meiotic cell division and later the second division produced the macrotetrad. This sequence has been interpreted as a sexual cycle. The zygote is formed by the fusion of two genetically identical gametes, each of which originated by the vegetative division of a single haploid nucleus. This type of sexual mechanism may occasionally give rise to variation because it provides an opportunity for chromosomal rearrangements to occur. However, if no such accidents occur, the zygote produced by this fusion is homozygous so no reassortment of genes is possible. Moreover, since the fusion occurs within a single cell, and since no mechanism was observed capable of permitting the sexual combination of free cells, it is impossible to obtain hybrids in the species studied.

It is possible that this represents a very early evolutionary type of sexual mechanism and that the heterogametic sexual forms are more advanced phylogenetically. Parthenogenesis, when it occurs in higher forms, may be a reversion to an older phylogenetic process.

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# CONSOLIDATION OF ELASTIC EARTH LAYERS

D. L. HOLL

*From the Engineering Experiment Station, Iowa State College*

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## INTRODUCTION

It is well known in engineering practice that the settlement of soils under loads is caused by a phenomenon called consolidation. This mechanism in many cases is identical with the process of squeezing water out of an elastic porous medium. Such settlement is very apparent in clays and sands saturated with water and is due to the gradual adaptation of the soil to the variable distribution of stresses in the supporting medium.

A simple mechanism to explain this process of consolidation was first proposed by Terzaghi.<sup>1</sup> He assumed that the volume consolidation  $\varepsilon$  was propagated throughout the medium in accordance with the same law as the "heat equation." Terzaghi applied this concept with remarkable success to the settlement of a column of soil under a constant load, when the water was constrained to flow axially along the column and find egress at the ends of the column. Biot<sup>2</sup> has generalized this consolidation theory to three-dimensional problems with variable loads. The present paper applies this analysis to saturated earth layers of finite and infinite depth. In the axially symmetric case several types of boundary conditions are considered.

## EQUATIONS OF EQUILIBRIUM

The equations for the elastic consolidation of a water-laden medium are

$$(1) \quad \left\{ \begin{array}{l} G\nabla^2 u + \frac{G}{1-2v} \frac{\partial \varepsilon}{\partial x} - \alpha \frac{\partial \sigma}{\partial x} = 0 \\ G\nabla^2 v + \frac{G}{1-2v} \frac{\partial \varepsilon}{\partial y} - \alpha \frac{\partial \sigma}{\partial y} = 0, \\ G\nabla^2 w + \frac{G}{1-2v} \frac{\partial \varepsilon}{\partial z} - \alpha \frac{\partial \sigma}{\partial z} = 0, \end{array} \right.$$

<sup>1</sup>K. Terzaghi, *Erdbaumechanik*, Leipzig (1925); "Principles of soil mechanics," *Engr. News Record* (1924); Also see *Proc. Int. Cong. for Appl. Mechanics*, p. 288, Delft 1924.

<sup>2</sup>M. A. Biot, "General Theory of Three-Dimensional Consolidation," *Jour. of Appl. Phys.*, Vol. 12, pp. 151-64; "Consolidation Settlement Under a Rectangular Load Distribution," *Jour. of Appl. Phys.*, Vol. 12, pp. 426-39; "Consolidation Settlement of a Soil with an Impervious Top Surface," *Jour. of Appl. Phys.*, Vol. 12, pp. 578-81, 1941.

$$(2) \quad \nabla^2 \epsilon = \frac{a^2}{c} \frac{\partial \epsilon}{\partial t},$$

where

$u, v, w$  = components of displacement in the supporting medium

$G, v$  = shear modulus and Poisson ratio of a completely consolidated medium

$\sigma$  = hydrodynamic excess pore pressure

$$\epsilon = \frac{\partial u}{\partial x} + \frac{\partial v}{\partial y} + \frac{\partial w}{\partial z} = \text{divergence of displacement vector or the consolidation per cubic unit of soil}$$

$$c = \frac{k}{a} \text{ coefficient of consolidation}$$

$k$  = coefficient of soil permeability

$$a = \frac{1 - 2v}{2G(1 - v)}, \text{ final compressibility}$$

$a$  = ratio of water egress per unit volume to the "total consolidation" per unit volume.

In deriving these equations, Biot has assumed (1) the medium is isotropic, (2) the stress-strain relationships are reversible under final equilibrium conditions, (3) the strains are small and (4) are linear functions of the stresses, (5) the fluid itself is incompressible but may contain air bubbles, and (6) the osmotic pressure causes the fluid to flow in accordance with Darcy's law. In defining the ratio  $a$ , the total consolidation includes the consolidation of the pores containing air bubbles as well as the liquid pores. The range of  $a$  is from zero to one. If  $a = 1$  the soil is completely saturated, and if  $a = 0$  the soil is completely dehydrated. In a completely saturated soil it is assumed that the initial load-bearing capacity of the soil is carried by the water, but at any subsequent time the load is continuously transferred to the soil skeleton.

#### AXIALLY SYMMETRIC LAYER

Consider a layer of soil of finite depth  $h$  supported on a rigid rock surface and infinite in extent in all horizontal directions. Adopting cylindrical coordinates with the origin in the surface plane  $z = 0$  and the Z axis directed downward into the layer, one has the equilibrium equations (1) in the form

$$G \nabla^2 u + \frac{G}{1 - 2v} \frac{\partial \epsilon}{\partial r} - a \frac{\partial \sigma}{\partial r} = 0,$$

$$(3) \quad G \nabla^2 w + \frac{G}{1-2v} \frac{\partial \epsilon}{\partial z} - a \frac{\partial \sigma}{\partial z} = 0,$$

where  $u, w$  are the radial and axial components of displacement and

$$\epsilon = \frac{\partial u}{\partial r} + \frac{u}{r} + \frac{\partial w}{\partial z}.$$

It is expedient to employ the operational form of the equations involving time by introducing the operator  $p$  for  $\partial/\partial t$ , and Eq. (2) becomes

$$(4) \quad \nabla^2 \epsilon = a^2 p \epsilon / c.$$

It is also assumed that the surface loads  $q(r)$  applied instantly at  $t = 0$  are of such a character that they can be represented by a Fourier-Bessel integral. One seeks a solution of Eqs. (3) and (4) for  $u, w$ , and such that the boundary conditions at the surfaces  $z = 0$  and  $z = h$  of the layer are satisfied.

It can be shown that the following is a suitable form of the solution.

$$(5) \quad \begin{aligned} u(r, z, p) &= -[\lambda A \cosh \zeta z + \lambda B \sinh \zeta z + C_1 (\cosh \lambda z + \lambda z \sinh \lambda z) \\ &\quad + C_2 \sinh \lambda z + C_3 (\sinh \lambda z + \lambda z \cosh \lambda z) + C_4 \cosh \lambda z] J_1(\lambda r), \\ w(r, z, p) &= [\zeta A \sinh \zeta z + \zeta B \cosh \zeta z + C_1 \lambda z \cosh \lambda z + C_2 \cosh \lambda z \\ &\quad + C_3 \lambda z \sinh \lambda z + C_4 \sinh \lambda z] J_0(\lambda r), \\ a\sigma(r, z, p) &= [2G \delta \frac{pa^2}{c} (A \cosh \zeta z + B \sinh \zeta z) \\ &\quad + 2G (\lambda C_1 \cosh \lambda z + \lambda C_3 \sinh \lambda z)] J_0(\lambda r), \end{aligned}$$

(6) where

$$\zeta^2 = \lambda^2 + pa^2/c,$$

$$\delta = \frac{1-v}{1-2v}.$$

The six constants of integration can be determined from the boundary conditions. Three of these conditions are

$$(7) \quad \begin{aligned} \sigma_z &= -q(r) \text{ at } z = 0, \\ \tau_{rz} &= 0 \quad \text{at } z = 0, \\ w &= 0 \quad \text{at } z = h. \end{aligned}$$

The first and second conditions state that the applied surface load is a normal loading with no surface-shearing forces. The third condition states that the supporting rock surface is rigid, allowing no axial movement. The three remaining conditions are indicated for the following cases.

(I) *Smooth impervious rock support and open top surface.* These conditions are

$$\tau_{rz} = 0 \text{ at } z = h,$$

$$(8) \quad \frac{\partial \sigma}{\partial z} = 0 \text{ at } z = h,$$

$$\sigma = 0 \text{ at } z = 0.$$

The last boundary condition indicates that the excess water pressure vanishes at the top surface, or the total hydrostatic pressure becomes the atmospheric pressure at this surface. This assumes that the surface load is applied on a porous flexible mat which permits a free egress of surface water. The first two of Eqs. (8) indicate that the rock surface  $z = h$  is smooth and impervious to the flow of water.

The values of five of the constants for this case are

$$(9) \quad \begin{aligned} B &= -A \tanh \zeta h, \\ C_1 &= -\frac{8pa^2}{c} A, \\ C_2 &= A \left( \zeta \tanh \zeta h - \frac{\delta pa^2}{\lambda c} \tanh \lambda h \right), \\ C_3 &= A \frac{\delta pa^2}{\lambda c} \tanh \lambda h, \\ C_4 &= A \left[ -\frac{\zeta \tanh \zeta h}{\tanh \lambda h} + \frac{\delta pa^2}{\lambda c} (1 + \lambda h \operatorname{sech} \lambda h \operatorname{csch} \lambda h) \right]. \end{aligned}$$

(II) *Smooth porous rock support and open top surface.* In this case the second of Eq. (8) is replaced by  $\sigma = 0$  at  $z = h$ , and the constants are

$$(10) \quad \begin{aligned} B &= -A (\coth \zeta h - \operatorname{sech} \lambda h \operatorname{csch} \lambda h), \\ C_1 &= -\frac{\delta pa^2}{\lambda c}, \\ C_2 &= A \left[ \zeta \coth \zeta h - \zeta \operatorname{sech} \lambda h \operatorname{csch} \zeta h - \frac{\delta pa^2}{\lambda c} \tanh \lambda h \right], \\ C_3 &= A \frac{\delta pa^2}{\lambda c} \tanh \lambda h, \\ C_4 &= -\zeta A (\coth \zeta h \coth \lambda h - 2 \operatorname{csch} \zeta h \operatorname{csch} \lambda h \\ &\quad + \coth \zeta h \operatorname{sech} \lambda h \operatorname{csch} \lambda h) + \frac{\delta pa^2}{\lambda c} A (1 + \lambda h \operatorname{csch} \lambda h \operatorname{sech} \lambda h). \end{aligned}$$

(III) *Smooth impervious rock support and impervious top surface.*

$$B = -A \tanh \zeta h,$$

$$(11) \quad C_1 = -\frac{\delta p \alpha^2}{\lambda^2 c} \zeta A \coth \lambda h \tanh \lambda h ,$$

$$C_2 = \zeta A \tanh \zeta h - \frac{\delta p \alpha^2}{\lambda^2 c} \zeta A \tanh \zeta h ,$$

$$C_3 = \frac{\delta p \alpha^2}{\lambda^2 c} \zeta A \tanh \zeta h ,$$

$$C_4 = -\zeta A \tanh \zeta h \coth \lambda h + \frac{\delta p \alpha^2}{\lambda^2 c} \zeta A \tanh \zeta h (\coth \lambda h$$

$$+ \lambda h \operatorname{csch}^2 \lambda h).$$

(IV) Smooth porous rock support and impervious top surface.

$$A = -B (\tanh \zeta h + \frac{\zeta}{\lambda} \operatorname{csch} \lambda h \operatorname{sech} \zeta h) ,$$

$$C_1 = \frac{\delta p \alpha^2}{\lambda^2 c} \zeta B ,$$

$$(12) \quad C_2 = \zeta B \left( \frac{\delta p \alpha^2}{\lambda^2 c} - 1 \right) ,$$

$$C_3 = -\zeta B \cdot \frac{\delta p \alpha^2}{\lambda^2 c} ,$$

$$C_4 = -\zeta B \left( \operatorname{sech} \zeta h \operatorname{csch} \lambda h - \coth \lambda h - \frac{\zeta}{\lambda} \operatorname{csch}^2 \lambda h \tanh \zeta h \right)$$

$$- \zeta B \frac{\delta p \alpha^2}{\lambda^2 c} (\lambda h \operatorname{csch}^2 \lambda h + \coth \lambda h) .$$

It is interesting to note that the limiting forms of these values (9) and (10), also (11) and (12), agree when  $h$  becomes indefinitely large, that is when the earth layer becomes a semi-infinite region. The values of these constants are somewhat more complicated when the rock support is rough. In this case the condition  $u = 0$  replaces the condition

$\tau_{rs} = 0$  at the surface  $z = h$ .

In order to complete any one of these cases, the remaining constant must be determined from the condition that the stress  $\sigma_s$  must balance the applied load  $q(r)$  at  $z = 0$ . The stresses are given by

$$\sigma_s = 2G \left( \frac{\partial w}{\partial z} + \frac{v\epsilon}{1-2v} \right) - \alpha \sigma ,$$

$$(13) \quad \begin{aligned} \sigma_r &= 2G\left(\frac{\partial u}{\partial r} + \frac{v\varepsilon}{1-2v}\right) - \alpha\sigma, \\ \sigma_\theta &= 2G\left(\frac{u}{r} + \frac{v\varepsilon}{1-2v}\right) - \alpha\sigma, \\ \tau_{rz} &= G\left(\frac{\partial w}{\partial r} + \frac{\partial u}{\partial z}\right). \end{aligned}$$

We shall take the surface loading to be

$$q(r) = \lambda Q(\lambda) J_0(\lambda r)$$

where  $Q(\lambda)$  will indicate various loading intensities. From the first of Eqs. (13) one finds

$$(14) \quad [\sigma_z]_{z=0} = -q(r) = -\lambda Q(\lambda) J_0(\lambda r).$$

Two cases arise according to whether  $\sigma$  vanishes or does not vanish at the surface. For I and II the identity in  $r$  in Eq. (14) leads to

$$(15) \quad A = \frac{-\lambda Q(\lambda)}{2G(\lambda^2 + \lambda C_4/A)},$$

where the ratio  $C_4/A$  is given by the last of Eqs. (9) and (10) for the two cases of an impervious and porous rock surface. In the cases III and IV the final constant can also be expressed in terms of  $\lambda$  and  $Q(\lambda)$  the loading intensity.

One observes that when  $\alpha = 0$ , equations (3) and (4) are the equilibrium equations of an elastic medium containing no excess water pressure, and (13) gives the corresponding stresses. Only the normal stresses in (13) are supplemented by this excess water pressure. By carefully examining the indeterminate forms which arise when  $\alpha \rightarrow 0$  in the solution given by Eq. (5), one can show that  $\sigma$  is identically zero everywhere and  $u$  and  $w$  agree with the solution obtained by Marguerre<sup>8</sup> for a layer on a smooth rock surface.

Since the solution is in operational form, one may conclude that the same limiting form of the solution is obtained when  $\alpha \neq 0$  and  $p \rightarrow 0$  as when  $p \neq 0$  and  $\alpha \rightarrow 0$ . However, it is known from operational calculus that the solution for  $t \rightarrow \infty$  is obtained by allowing  $p \rightarrow 0$ . This permits one to state that the final state of consolidation is the same as the instantaneous state of the same dehydrated earth under purely elastic deformation.

#### SURFACE DEFLECTION OF AXIALLY SYMMETRIC LAYER

Consider only cases I and II. From Eq. (5) one finds that the surface deflection is

$$[w]_{z=0} = (\zeta B + C_2) J_0(\lambda r)$$

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<sup>8</sup> K. Marguerre, "Spannungsverteilung und Wellenausbreitung in der Kontinuierlich gestützten Platte," Ingenieur-Archiv, 4: pp. 332-53, 1933.

$$(16) \quad \begin{aligned} &= -\frac{\delta p \alpha^2}{\lambda c} A \tanh \lambda h J_0(\lambda r) \\ &\quad \frac{apa^2}{c} Q(\lambda) \tanh \lambda h J_0(\lambda r) \\ &\quad \frac{\delta p \alpha^2}{c} \lambda^2 + \lambda C_4/A \end{aligned}$$

This is the operational form of the surface deflection when subjected to a load  $q(r) = \lambda Q(\lambda) J_0(\lambda r)$ . For an impervious smooth surface at  $z = h$ , equation (16) becomes

$$(17) \quad [w]_{z=0} = \frac{apa^2}{c} \frac{\frac{Q(\lambda) \tanh \lambda h J_0(\lambda r)}{\lambda^2 - \lambda \zeta \coth \lambda h \tanh \zeta h + \frac{1}{c}}}{\frac{\delta p \alpha^2}{c} (1 + \lambda h \operatorname{sech} \lambda h \operatorname{csch} \lambda h)}$$

The limiting values of this surface deflection at  $t = 0$  and at  $t = \infty$  (i.e.,  $p \rightarrow \infty$  and  $p \rightarrow 0$ ) are

$$(18) \quad w(r, 0, 0) = \frac{Q(\lambda) \sinh^2 \lambda h J_0(\lambda r)}{2G (\lambda h + \sinh \lambda h \cosh \lambda h)},$$

$$(19) \quad w(r, 0, \infty) = \frac{(1-v) Q(\lambda) \sinh^2 \lambda h J_0(\lambda r)}{G (\lambda h + \sinh \lambda h \cosh \lambda h)}.$$

This shows that the final displacement is  $2(1-v)$  times the initial displacement for any type of loading  $q(r)$ . Moreover, the same limiting displacements given by (18) and (19) which are deduced for Case I also hold for Case II, i.e., when the smooth surface is porous. Although the initial and final displacements are the same, the rate at which settlement occurs differs. In the latter case, where the rock surface is porous, the settlement is more rapid.

#### SEMI-INFINITE LAYER

As an example of the previous analysis, consider the case of a uniformly distributed circular load applied at  $t = 0$  to the upper surface of a semi-infinite earth containing imprisoned water. This loading may be represented by the superposition of an infinite number of loads of the type  $Q(\lambda) J_0(\lambda r)$  if

$$Q(\lambda) = P J_1(\lambda R) / \pi \lambda R, \quad \text{where } P = \pi q_0 R^2$$

is the total load over the circle of radius  $R$ . Introducing this loading and allowing  $h$  to become indefinitely large, one finds from (17)

$$(20) \quad [w]_{z=0} = \frac{aP}{\pi} \int_0^\infty \frac{p \alpha^2 J_1(\lambda R) J_0(\lambda r) d\lambda}{c \lambda R (\lambda^2 - \lambda \zeta + \delta p \alpha^2 / c)}$$

The deflection at any time  $t$  is

$$(21) \quad [w(t)]_{z=0} = \frac{P}{2\pi G R} \int_0^c J_1(\lambda R) J_0(\lambda r) F(v, \lambda \sqrt{ct}/a) d\lambda/\lambda,$$

where

$$(22) \quad F(v, \xi) = 1 - v(1 - e^{-\beta\xi^2}) - (1 - v) \operatorname{Erf} \xi - ve^{-\beta\xi^2} \operatorname{Erf}(\xi \sqrt{1-\beta}),$$

where  $\xi^2 = \lambda^2 ct/a^2$ ,  $\beta = \frac{1-2v}{(1-v)^2}$ ,

and  $\operatorname{Erf} x = \frac{2}{\sqrt{\pi}} \int_0^\infty e^{-s^2} ds.$

This latter integral is the "error function" or probability integral and is tabulated.

By shrinking the circular load to a point load, one finds that (21) becomes

$$(23) \quad [w(t)]_{z=0} = \frac{P}{4\pi G} \int_0^\infty J_0(\lambda r) F(v, \xi) d\lambda.$$

From this result, one can derive the plane strain case by summing the deflections due to an infinitely long line load. This yields

$$(24) \quad [w]_{z=0} = \frac{P}{2\pi G} \int_0^\infty F(v, \xi(\lambda)) d\lambda \int_0^\infty J_0(\lambda \sqrt{x^2+y^2}) dy \\ = \frac{P}{2\pi G} \int_0^\infty F(v, \xi(\lambda)) \cos \lambda x d\lambda/\lambda.$$

This result may be employed to obtain the result derived by Biot<sup>2</sup> for a long rectangular load of width  $2L$ ,

$$(25) \quad [w]_{z=0} = \frac{P}{2\pi G} \int_0^\infty F(v, \xi(\lambda)) d\lambda/\lambda \int_{-L}^L \cos \lambda(x-x') dx' \\ = \frac{P}{2\pi G} \int_0^\infty F(v, \xi(\lambda)) [\sin \lambda(x+L) - \sin \lambda(x-L)] d\lambda/\lambda^2.$$

This integral does not exist. However, by subtracting the initial displacement, i.e., Eq. (25) with  $F(v, 0)$ , we may examine the subsequent settlement which Biot has considered.

## THREE-DIMENSIONAL SOLUTION

Consider a semi-infinite earth supporting a double wave load of the type

$$(26) \quad q(x, y) = Q(\lambda, \kappa) \cos \lambda x \cos \kappa y.$$

The problem is to find a solution of (1) and (2) such that  $u$ ,  $v$ ,  $w$ , and  $\sigma$  vanish at infinity, with  $\sigma = \tau_{xz} = \tau_{yz} = 0$  at  $z = 0$  and  $\sigma_z = -q$  at  $z = 0$ .

This solution is

$$(27) \quad \begin{aligned} u &= [\lambda C_1 e^{-\gamma z} - \lambda C_2 (1 - \mu z) e^{-\mu z} + \lambda C_3 e^{-\mu z}] \sin \lambda x \cos \kappa y, \\ v &= [\kappa C_1 e^{-\gamma z} - \kappa C_2 (1 - \mu z) e^{-\mu z} + \kappa C_3 e^{-\mu z}] \cos \lambda x \sin \kappa y, \\ w &= [\gamma C_1 e^{-\gamma z} + \mu^2 C_2 z e^{-\mu z} + \mu C_3 e^{-\mu z}] \cos \lambda x \cos \kappa y, \\ \alpha \sigma &= \left[ -2G\delta C_1 \frac{pa^2}{c} e^{-\gamma z} + 2G\mu^2 C_2 e^{-\mu z} \right] \cos \lambda x \cos \kappa y, \\ \mu^2 &= \lambda^2 + \kappa^2, \\ \text{where } \gamma^2 &= \mu^2 + pa^2/c, \\ C_3 &= C_1 \left( \frac{\delta pa^2}{\mu^2 c} - \frac{\gamma}{\mu} \right), \\ (28) \quad C_2 &= C_1 \frac{\delta pa^2}{\mu^2 c}. \end{aligned}$$

This is the operational form of the solution in terms of the undetermined constant  $C_1$  for a semi-infinite earth with imprisoned water which has free egress at the top surface. From the first of Eq. (13) and the identity arising from

$$[\sigma_z]_{z=0} = -Q(\lambda, \kappa) \cos \lambda x \cos \kappa y$$

one finds

$$(29) \quad C_1 = \frac{(1-2v)Q(\lambda, \kappa)(\mu\gamma + \mu^2 + \delta pa^2/c)}{(2Gpa^2/c)(\mu^2 + pa^2/c)}$$

The surface deflection is

$$[w]_{z=0} = \frac{\delta pa^2}{\mu c} C_1 \cos \lambda x \cos \kappa y.$$

Consider the example of a load  $P$  uniformly distributed over a rectangular area  $2l$  by  $2m$ , then

$$Q(\lambda, \kappa) = \frac{2P}{\pi lm} \frac{\sin \lambda l \sin \kappa m}{\kappa \lambda}$$

From the principle of superposition, one may show that the surface deflection is given by

$$(30) [w]_{z=0} = \frac{2P}{\pi l m G} \int_0^\infty \int_0^\infty F(v, \mu \sqrt{ct}) \cos \lambda x \cos \kappa y \sin \lambda l \sin \kappa m \frac{d\lambda dk}{\kappa \lambda \mu}.$$

In a subsequent paper some graphical results will be shown for particular types of loads in the axially symmetric case and the three-dimensional case in rectangular coordinates.

## TWO NEW PERUVIAN TINGITIDAE (HEMIPTERA)

CARL J. DRAKE, Iowa State College, Ames, Iowa

AND

E. J. HAMBLETON, Sociedad Nacional Agraria, Lima, Peru, S. A.

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The present paper contains the descriptions of two new lace bugs from Peru and notes on another species found breeding on cotton. The holotype and allotype are in the Drake Collection and paratypes in the collection of both authors.

### *Monanthia senta*, sp. nov.

Moderately large, ovate, yellowish brown, with a few brown to dark fuscous markings. Head reddish brown, with five, rather long, slender, porrect, testaceous spines, the apical half of spines generally dark fuscous with the tips testaceous; antennae slender, testaceous, indistinctly hairy, the terminal segment largely black-fuscous; segment I short, slightly longer and slightly stouter than II; III very slender, nearly four times as long as IV; IV subclavate, with long hairs; bucculae broad, closed in front, areolate. Rostral channel deep, rather narrow on mesosternum, very abruptly widened and extremely broad on metasternum, there with the laminae of the sides strongly converging posteriorly and entirely open behind. Orifice indistinct.

Pronotum convex, tricarinate, subtruncate in front, median carina composed of one row of small areolae, beset with small spines above, in front slightly elevated and there with the collar slightly inflated so as to form a very small hoodlike structure; collar distinct, reticulate; paranota very broad, strongly reflexed and resting on the dorsal surface of pronotum, each with outer margin touching the median carina and with two strongly raised, narrow, moderately high, inflated, sharply arched elevations, the median nervure of each elevation beset with short spines; lateral carinae exposed on triangular process, there slightly arched and divaricating posteriorly. Elytra broad, testaceous, some of the nervelets brown to dark fuscous; costal area broad, biseriate, with the marginal nervure very pale, biseriate, sometimes with an extra areolae in widest part, the areolae very large, irregular in both size and arrangement, the transverse nervures thick and brown to dark fuscous; subcostal area very broad, finely areolate; discoidal area with large, reversed C-shaped area at the apex extending deeply into subcostal area, there with the boundary at base and apex sharply raised.

Holotype (male), allotype (female), 14 paratypes, collected on a woody shrub, Sullena and Paila, Peru, May 10, 1940, E. J. Hambleton.

The four, high and sharply raised projections on pronotum (part of paranota) and the strongly developed apical portion of nervure clos-

ing discoidal area, which forms a peculiar, reversed C-shaped mark, are distinguishing characters. This species is not easily confused with other members of *Monanthia* from the Western Hemisphere.

*Corythaica costata* Gibson

Common on cotton and widely distributed in Peru; known also from Ecuador. Separated readily from *C. cyathicollis* Costa by the small bulla on the nervure (beyond middle) separating discoidal and sutural areas and the uniseriate costal area. *C. cytharina* (Butler) from Galapagos Island, is a very closely related species. The latter seems to be distinctly smaller and has the elytra slightly more constricted beyond the middle; the hood, paranota, carinae, and other structures are much smaller.

Although the genus *Corythaica* Stål, 1873, is a very distinct genus, it has been much confused in the literature. The genera *Typonotus* Uhler, 1898, and *Leptotingis* Monte, 1938, are both synonyms of *Corythaica*. *Leptotingis* (= *Corythaica*) *umbrosa* Monte is a good species and a typical member of the genus *Corythaica*.

Long- and short-winged specimens of *C. umbrosa* (Monte) are at hand from Paraguay and Brazil.

*Corythucha nocentis*, sp. nov.

Small, whitish testaceous, with fuscous markings. Antennae pale testaceous, moderately long, beset with long, stiff hairs. Rostrum extending almost to end of sulcus, black at apex; laminae whitish, areolate. Body beneath black. Legs slender, pale testaceous, the tarsi brown.

Pronotum pale stramineous, slightly convex, indistinctly punctate; triangular process small, mostly white, areolate behind; lateral carinae short, scarcely extending forward beyond triangular process, raised anteriorly and moderately high in front, areolate; median carinae moderately elevated, arched above, about one-half as long and nearly one-half as high as hood, areolate; hood moderately large, strongly constricted at the middle, narrowed in front, inflated and the areolae at crest of hind portion mostly infuscate, extending back over disc of pronotum. Elytra distinctly constricted at the middle, not narrowed posteriorly, whitish testaceous with a transverse band near the base and another broader one at the apex dark fuscous, the latter with two large, clear cells, some of the areolae in both bands partly clear; tumid elevation moderately large, with few fuscous spots; costal area broad, triseriate.

Length, 2.70 mm.; width, 1.60 mm.

Holotype (male), allotype (female) and many paratypes, Dec. 14, 1940, Canete, Peru.

This species resembles *C. marmorata* Uhler in general appearance, but less marmorate and with different hood and carinae. *C. morrilli* O. & D. is a more elongate species. The paranota are large, whitish and usually with a small fuscous spot near the middle. The outer margins of elytra, paranota and sides of hood are beset with sharp, mostly black-tipped spines. The male is usually a little smaller than the female.

# SEASONAL FOOD PREFERENCE TRENDS OF EASTERN RUFFED GROUSE IN IOWA AS SHOWN BY DROPPING ANALYSIS<sup>1</sup>

EMMETT B. POLDERBOER

From the Entomology and Economic Zoology Section, Iowa Agricultural Experiment Station, and the Fish and Wildlife Service, United States Department of the Interior

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Short articles have appeared from time to time in various ornithological periodicals regarding the food habits of the ruffed grouse. Most of them have dealt with direct observations, and stomach and crop analysis.

From August, 1938, until March, 1940, the writer spent considerable time in the field investigating the cover requirements of the ruffed grouse (*Bonasa umbellus umbellus*) in northeastern Iowa. Incidental to the cover research, droppings were collected and analyzed for food material content. Assistance and guidance in this research was given by Dr. G. O. Hendrickson, Department of Zoology and Entomology, and Mr. Thomas G. Scott, United States Fish and Wildlife Service. Identification of insect remains was facilitated by the assistance of Dr. H. H. Knight, Department of Zoology and Entomology.

Droppings were found at night roosts and day time loafing roosts which were located by searching under hazelnut brush and dogwood brush in the vicinity of scratch beds and dust baths. Night roosts contained from 30 to 70 individual fecal pellets to the roost sample. Loafing roosts contained from 10 to 15 pellets. Small samples containing less than 10 pellets were not collected.

Collected fecal material was kept separately wrapped in small sacks. Preparatory to examination the pellets were soaked in warm water until they could easily be broken down by passing tap water over them. A number 40 sieve was used in the separation process. Ureats, fine matter, and solubles passed through the sieve and left larger fragments that could be identified by comparison with a control collection with the aid of a 10-power binocular microscope. Before washing through the sieve the pellets were examined for the presence of anthers and other small materials easily washed through the sieve.

A total of 176 samples was examined. The numbers of samples for the seasons were as follows: 33 spring, 26 summer, 51 fall, and 66 winter. Each individual pellet in a sample was examined; each food item found in a pellet was given the value of one frequency, and percentages were derived from the sums of the frequencies of all food items in the sample. For the entire season, percentages were derived from the cumulative percentages of all samples examined. These figures may not give the

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true percentages of volume or weight of foods taken, and soft-bodied animals or finely digested food matter that is easily destroyed in digestion may not be represented in this analysis as they would be in crop analysis and stomach analysis procedures. The chief value of this method is in showing seasonal trends of food habits among a game species too scarce to be killed for stomach and crop analysis.

The seasonal food trends in order of percentage of seasonal frequency are shown in Table 1. Percentages of animal foods taken in each of the

TABLE 1  
IOWA RUFFED GROUSE FOODS IN ORDER OF SEASONAL FREQUENCY

	Percentage
<i>Spring foods</i>	
Miscellaneous buds .....	30.5
Hog peanut leaves ( <i>Amphicarpa monoica</i> ) .....	11.1
Grass leaves (Gramineae) .....	9.3
Hazelnut catkins ( <i>Corylus americana</i> ) .....	9.1
Choke cherry buds ( <i>Prunus virginiana</i> ) .....	6.3
Dandelion leaves ( <i>Taraxacum</i> ) .....	6.0
Strawberry leaves ( <i>Fragaria</i> ) .....	5.1
Birch buds ( <i>Betula lutea</i> ) .....	4.1
Strawberry fruit .....	3.5
Miterwort leaves ( <i>Mitella diphylla</i> ) .....	3.1
Windflower leaves ( <i>Anemone</i> ) .....	2.2
Oak buds ( <i>Quercus</i> ) .....	2.1
Elm seed ( <i>Ulmus</i> ) .....	1.4
Bees (Apidae) .....	1.3
Maple seeds ( <i>Acer</i> ) .....	.5
Sweet Cicely seeds ( <i>Osmorhiza claytoni</i> ) .....	1.1
Spiders (Araneida) .....	.9
Grasshoppers (Acrididae) .....	.8
Raspberry leaves ( <i>Rubus</i> ) .....	.8
Ichneumon fly (Ichneumonidae) .....	.4
Saxifrage leaves ( <i>Sullivantia Sullivantii</i> ) .....	.3
Moss (Musci) .....	.2
<i>Summer foods</i>	
Beetles (Carabidae) .....	10.0
Bluegrass leaves ( <i>Poa</i> ) .....	9.1
Miscellaneous leaves .....	8.0
Beetle (Curculionidae) .....	7.4
Acorn ( <i>Quercus</i> ) .....	7.2
Tree cricket ( <i>Pterophylla camellifolia</i> ) .....	6.8
Gravel .....	5.5
Raspberry fruit .....	4.0
Bug (Pentatomidae) .....	3.7
Undetermined beetles (Coleoptera) .....	3.2
Dogwood seeds ( <i>Cornus</i> ) .....	2.8
Sandcherry seeds ( <i>Prunus pumila</i> ) .....	2.6
Cockroach ( <i>Parcoblatta pennsylvanica</i> ) .....	2.6
Trefoil leaves ( <i>Desmodium</i> ) .....	2.4
Miscellaneous buds .....	2.2
Ants (Formicidae) .....	2.1
Beetles (Lucanidae) .....	2.1
Beetles (Chrysomelidae) .....	1.2
Sedge seeds ( <i>Carex</i> ) .....	1.1

	Percentage
Crickets ( <i>Gryllus</i> ) .....	1.1
Grasshoppers .....	1.1
Spider .....	1.0
Pigeon grass seeds ( <i>Setaria</i> ) .....	1.0
Blueberry fruits ( <i>Vaccinium canadense</i> ) .....	1.0
Ichneumon fly (Ichneumonidae) .....	.9
Undetermined Hymenoptera .....	.8
Beetle larvae (Coleoptera) .....	.7
Beetles (Tenebrionidae) .....	.7
Sweet Cicely seeds .....	.7
Bluegrass seeds ( <i>Poa</i> ) .....	.6
Hazelnut fruit ( <i>Corylus americana</i> ) .....	.5
Wasps (Chalcidae) .....	.5
Wasps (Braconidae) .....	.5
Crane flies (Tipulidae) .....	.5
Poplar buds ( <i>Populus</i> ) .....	.5
Beetles (Platystomidae) .....	.4
Self-heal leaves ( <i>Prunella vulgaris</i> ) .....	.4
Lace bugs (Tingitidae) .....	.4
Beetles (Scarabaeidae) .....	.4
Fly larvae (Diptera) .....	.4
Beetles (Otiorhynchinae) .....	.4
Harvestmen (Phalangidae) .....	.3
Choke cherry fruit .....	.3
Honeysuckle fruit ( <i>Lonicera</i> ) .....	.3
Nine-bark seeds ( <i>Physocarpus</i> ) .....	.2
Pigweed seeds ( <i>Amaranthus spinosus</i> ) .....	.2
Poison ivy seeds ( <i>Rhus toxicodendron</i> ) .....	.2
Solomon's seal seeds ( <i>Polygonatum commutatum</i> ) .....	.1
Beetles (Coccinellidae) .....	.1
Mouse bones and hair ( <i>Peromyscus</i> ) .....	.1
Feathers (Aves) .....	.1
<b>Fall foods</b>	
Acorn .....	21.5
Miscellaneous buds .....	11.9
Hazel catkins .....	10.9
Miscellaneous leaves .....	7.8
Sumac seeds ( <i>Rhus</i> ) .....	7.5
Sandcherry fruit .....	5.9
Gravel .....	4.0
Tree crickets .....	3.7
Hazelnut buds .....	3.4
Poplar buds .....	2.8
Bluegrass .....	2.5
Oak buds .....	2.0
Birch buds .....	2.0
Chrysomelid beetles .....	1.3
Dandelion leaves .....	1.2
New Jersey tea seeds ( <i>Ceanothus</i> ) .....	1.2
Mammal hair (Mammalia) .....	1.1
Grape fruits ( <i>Vitis</i> ) .....	1.0
Dogwood seeds .....	1.0
Honeysuckle seeds .....	1.0
Feathers .....	1.0
Undetermined bug (Hemiptera) .....	.8
Sedge seeds .....	.5
Carabid beetles .....	.5

	Percentage
Clover leaves ( <i>Trifolium repens</i> ) .....	.4
Red Haw fruits ( <i>Crataegus</i> ) .....	.4
Bugs (Miridae) .....	.4
Self-heal leaves .....	.3
Polygonum seeds ( <i>Polygonum convolvulus</i> ) .....	.3
Nine-bark seeds .....	.3
Ichneumon fly .....	.2
Undetermined beetles .....	.2
Mites (Acarina) .....	.2
Spiders .....	.2
Lead plant seeds ( <i>Amorpha canescens</i> ) .....	.2
Hog peanut seeds .....	.2
Trefoil seeds .....	.2
<b>Winter foods</b>	
Miscellaneous buds .....	23.0
Smooth sumac seeds ( <i>Rhus glabra</i> ) .....	20.7
Acorn .....	13.4
Pine needles .....	11.4
Staghorn sumac seeds ( <i>Rhus typhina</i> ) .....	8.5
Birch catkins .....	7.1
Poison ivy seeds .....	6.3
Miterwort leaves .....	3.4
Poplar buds .....	3.1
Oak buds .....	1.1
Miscellaneous leaves .....	.7
Honeysuckle seeds .....	.7
Gravel .....	.5
Lamb's-quarters seeds ( <i>Chenopodium album</i> ) .....	.1

seasons are as follows: winter, 0.0; spring, 3.4; summer, 49.4; and fall, 9.6. With the exception of small quantities of mouse hair and bones (*Peromyscus*), and feathers, possibly taken by preening, the entire animal food content consisted of arthropods of the orders *Arachnida* and *Insecta*. The high content of animal matter in the summer samples (July, August, and September) probably bears some relationship to samples from young birds whose droppings could not be differentiated from those of adults in August and September.

Gravel was very little in evidence in the winter and spring, but quite prevalent in the summer and fall samples. Gravel appeared in larger amounts when animal matter was present in the droppings. Winter

TABLE 2  
SEASONAL VARIATIONS IN MAJOR FOOD GROUP CONSUMPTION IN PERCENTAGE

Fod Groups.....	Spring	Summer	Fall	Winter	Entire Year
Buds.....	43.0	2.7	22.1	27.2	23.8
Leaves.....	38.1	19.8	12.2	15.5	21.4
Seeds and fruits.....	6.4	22.6	41.2	49.7	29.9
Catkins.....	9.1	0.0	10.9	7.1	6.8
Animal matter.....	3.4	49.4	9.6	0.0	15.6
Gravel.....	0.0	5.5	4.0	0.5	2.5

and spring droppings contained many twig fragments taken with buds. December, January, February, and March samples contained sumac seeds and acorn fragments that may have acted as masticating agents in the place of gravel. Percentages of plant foods taken were as follows: fall 86.4, winter 99.5, spring 96.6, and summer 45.1. Major groups of plant items such as buds, catkins, leaves, and fruits are shown in Table 2.

Table 2 demonstrates that seeds and fruits are especially prominent in the grouse diet during the fall and winter months (October 1 to March 31). The prominent seeds and fruits of fall and winter are acorns and sumac seeds. Leaves occur in greatest abundance in spring and summer. Dominant as leaf foods during these seasons were hog peanut, bluegrass, dandelion, and wild strawberry leaves. The highest frequency of buds, contrary to expectations, occurred in the spring months with 36 per cent of the total of 43 per cent taken during April when the buds were beginning to swell and open.



# THE DISSIMILATION OF GLUCOSE BY CHAETOMIUM FUNICOLA CKE.

## II. Influence of Some Modifications in the Composition of Czapek-Dox Medium on the Rate of Glucose Dissimilation.<sup>1</sup>

G. SEMENIUK<sup>2</sup>

From the Botany and Plant Pathology Section, Iowa Agricultural Experiment Station

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### INTRODUCTION

In a previous study (4) *Chaetomium funicola* Cke. was found to dissimilate glucose slowly in Czapek-Dox medium under conditions of gentle or vigorous agitation of the medium. To determine the influence of the composition of this medium on the rate of glucose dissimilation, the concentrations of glucose and  $\text{KH}_2\text{PO}_4$ , the initial pH, and the source of inorganic nitrogen were studied. The general fate of the dissimilated glucose was followed through yields of mycelium and the pH changes in the medium. The results of these studies are presented.

The cultural behavior of *C. funicola* has not been previously reported. Furthermore, other species of *Chaetomium* have been scarcely investigated. Tschudy (8) found nine species of *Chaetomium* to develop on an agar medium with pH 4.2 to 11.0, with the best development occurring on the alkaline side and no growth at pH 3.0, while Dickson (1) reported the development of seven species in a linear manner on an agar medium containing different concentrations of glucose, starch, and potassium phosphate, with no formation of staling products.

### EXPERIMENTAL

#### MATERIALS AND METHODS

The same culture of *C. funicola* was used here as in the previous study (4). Except for changes in concentration of glucose and certain inorganic salts, which will be indicated under each experiment, the composition of Czapek-Dox medium was as follows: 1 liter distilled water, 50.0 gm. Pfanstiehl's technical glucose, 2.0 gm.  $\text{NaNO}_3$ , 1.0 gm.  $\text{KH}_2\text{PO}_4$ , 0.5 gm. KCL, 0.5 gm.  $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$  and 0.01 gm.  $\text{FeSO}_4 \cdot 7\text{H}_2\text{O}$ . Pyrex Erlenmeyer flasks of 250 cc. capacity were used as culture flasks. Forty cc. of liquid medium were introduced into each flask in the first and fourth experiments, and 35 cc. were introduced in the second and third. To avoid any expected change in the medium during sterilization

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at 15 lbs. steam pressure for 15 minutes, certain components of the medium were sterilized separately. With this procedure different components of the medium were prepared of such concentration that when combined with the major portion of the medium after sterilization and after the aseptic addition of a definite volume of ascospore suspension (usually 5 cc.), the final desired concentration of ingredients was obtained.

Non-absorbent cotton plugs served as stoppers for the culture flasks. Culture flasks were maintained on flat horizontal surfaces. The temperature ranged from 25° to 30°C. Cultures were decanted and filtered by suction through washed, dried, and weighed pieces of percale cloth. The mycelium was washed several times with hot distilled water in the culture flask and on the filter. Drying of the mycelium to constant weight was effected at approximately 70°C. for several days followed by maintenance over concentrated H<sub>2</sub>SO<sub>4</sub> in a water-pump evacuated desiccator. The filtrates were made to 200 cc. volume and analyzed immediately.

Residual glucose in the filtrates was determined by the Bertrand modification of the Munson-Walker method (3) in experiments 1 and 4, and in experiments 2 and 3 by the Shaffer-Hartmann copper-iodate method as first modified and adapted to a semi-micro scale by Somogyi (6) and subsequently further modified by Schaffer and Somogyi (5), Harding and Downs (2), and Van der Plank (9). The solutions were prepared according to Van der Plank with the exception of the standard sodium thiosulphate solution, which was used as 0.005 N. This latter solution was prepared as recommended by Stiles, Peterson, and Fred (7). The starch solution was prepared as suggested by Willard and Furman (10) and the necessary precautions observed. Glucose values corresponding to the quantity of reduced Na<sub>2</sub>S<sub>2</sub>O<sub>3</sub> were read from an experimentally determined linear relationship. Hydrogen-ion concentration readings were made on the diluted filtrates with a Coleman glass electrode apparatus.

## RESULTS

### VARYING THE INITIAL GLUCOSE CONCENTRATION OF THE MEDIUM

Cultures were prepared without separate sterilization of the ingredients. The means of analyses of duplicate cultures after different periods of development are contained in Table 1 and are presented graphically in Figures 1, 2, and 3. The following observations were made from these data: (1) Glucose disappearance occurred in an approximately linear manner (Fig. 1). (2) The rate of glucose disappearance was greater at the higher concentrations of glucose than at the lower. (3) The disappearance of glucose from the medium when present in approximately 2.8 per cent initial concentration was nearly complete on the twenty-sixth day of fungus development. (4) Increases in mycelial weight on different initial concentrations of glucose were approximately the same for the first 17 days of fungus development. After this time further increases in

TABLE 1

EFFECT OF CONCENTRATION OF GLUCOSE IN CZAPEK-DOX MEDIUM ON GLUCOSE DISSIMILATION BY *Chaetomium funicola*

DAYS OF FUNGUS DEVELOPMENT	INITIAL PERCENTAGE GLUCOSE CONCENTRATION IN MEDIUM				
	2.8	5.5	7.5	10.1	13.5
MILLIGRAMS GLUCOSE PER 1 CC. OF CULTURE MEDIUM					
0	28.7	55.2	75.5	101.2	135.6
7	24.4	47.4	75.0	92.2	127.4
17	5.1	23.3	50.3	72.1	95.6
26	0.3	20.5	42.4	59.1	85.8
47	0	0	13.5	34.5	49.3
PH OF CULTURE MEDIUM					
0	4.72	4.95	4.58	4.77	4.61
7	6.29	6.20	6.10	6.22	6.35
17	7.87	7.52	7.63	7.71	7.84
26	7.62	7.15	7.10	7.46	7.35
47	8.70	8.04	6.46	6.25	6.28
MYCELIUM FORMED, MILLIGRAMS					
7	105.8	130.6	75.1	120.5	141.9
17	339.1	332.6	378.9	326.8	327.5
26	347.5	409.0	428.6	649.2	568.2
47	279.4	620.0	753.7	752.2	945.7

mycelial weight occurred only in cultures containing initial glucose concentrations of approximately 5.5 per cent and greater (Fig. 2). (5) Autolysis of the mycelium occurred in cultures initially containing approximately 2.8 per cent glucose but only when the glucose content in the medium was reduced to a low level. (6) Progressive change of pH toward the alkaline side occurred during the initial 17 days of fungus development at all concentrations of glucose used, followed by a decrease (Fig. 3). This decrease was temporary and small in cultures initially containing approximately 2.8 and 5.5 per cent glucose but was prolonged and greater in cultures containing higher concentrations of glucose. The resumed rise in pH following the temporary decrease in the cultures containing the lower concentrations of glucose was attributed to the autolytic processes taking place in these cultures.

#### VARYING THE INITIAL pH OF THE MEDIUM

Cultures containing nearly 10 per cent glucose were prepared. Calculated quantities of separately sterilized hydrochloric acid and sodium hydroxide solutions were added to the sterile medium. The means of analyses of duplicate cultures (Table 2) showed that the fungus failed to grow at an initial pH of 2.12 and grew only slightly when the initial pH was 2.90. Considerably more growth of *C. funicola* occurred at higher initial pH values. Reduced growth was encountered again at pH 8.68,

TABLE 2  
EFFECT OF INITIAL HYDROGEN-ION CONCENTRATION OF CZAPEK-DOX MEDIUM ON GLUCOSE DISSIMILATION BY *Chaetomium funicola*

DAYS OF FUNGUS DEVELOPMENT	INITIAL pH CULTURE MEDIUM						MILLICELUM FORMED, MILLIGRAMS		
	2.12	2.90	4.03	4.98	6.05	7.20	7.78	8.15	8.68
- MILLIGRAMS GLUCOSE PER 1 CC. OF CULTURE MEDIUM									
0	87.8	88.2	86.8	87.3	90.0	87.8	87.6	88.5	87.1
10	87.76	85.26	73.71	77.26	77.20	76.74	84.72	84.28	85.37
23	87.88	86.62	47.60	62.17	53.31	35.60	65.77	52.11	69.83
pH OF CULTURE MEDIUM									
10	2.50	3.25	7.01	6.92	6.89	6.98	7.02	7.41	7.47
23	2.55	4.45	7.28	6.86	7.34	6.78	7.97	8.05	8.15
MILLICELUM FORMED, MILLIGRAMS									
10	0.0	27.5	211.8	195.8	183.1	173.8	99.8	45.9	16.3
23	0.0	33.0	410.7	270.6	355.9	521.5	208.6	383.6	180.2

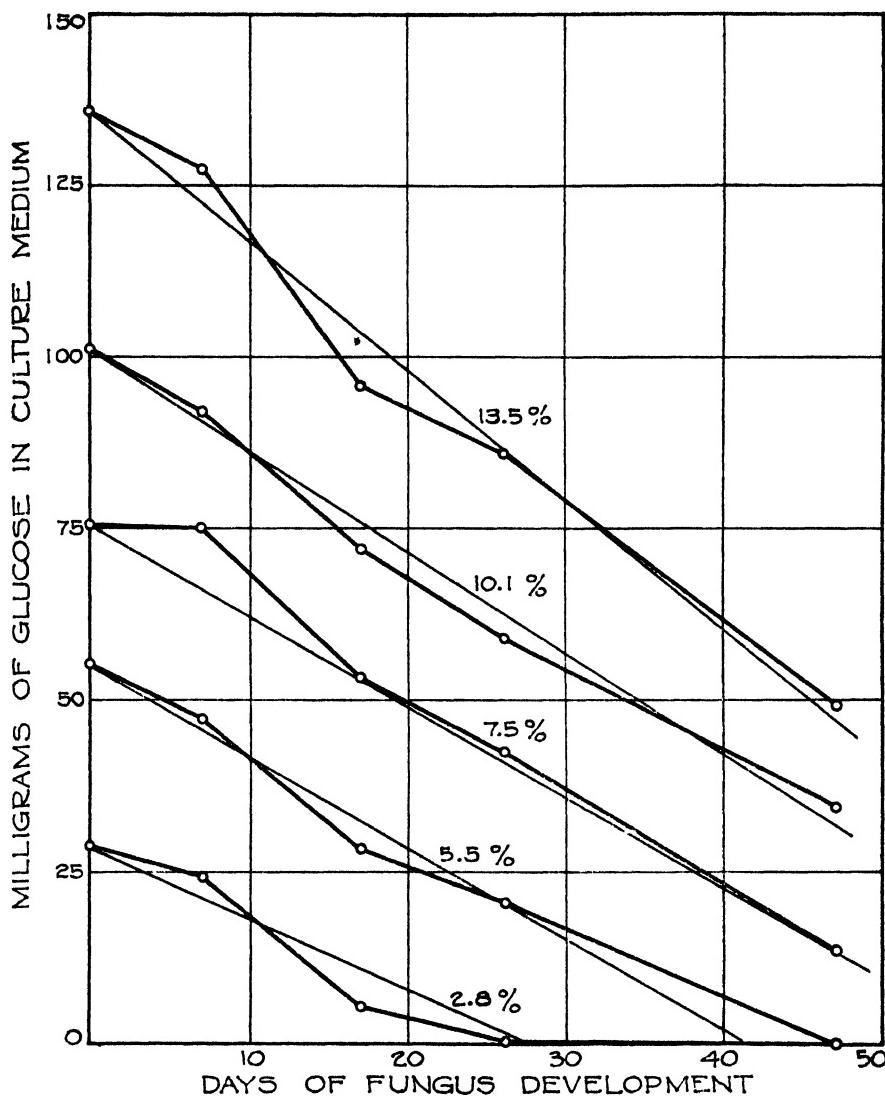


FIG. 1. Glucose remaining in Czapek-Dox medium containing different initial glucose concentrations after development of *Chaetomium funicola*.

which was the highest initial pH value tried. Tschudy (8) found pH 11.0 was the upper limit for growth of many species of *Chaetomium*, but in this test *C. funicola* was not included. He also found pH 3.0 was the lower limit for growth and the alkaline side yielded the optimum growth of these fungi. In the present experiment optimum growth of *C. funicola* after 10 days of development occurred at the initial pH value of 4.03, while after 23 days of development, optimum growth occurred at the initial pH of 7.20. In this latter period of development, however, three

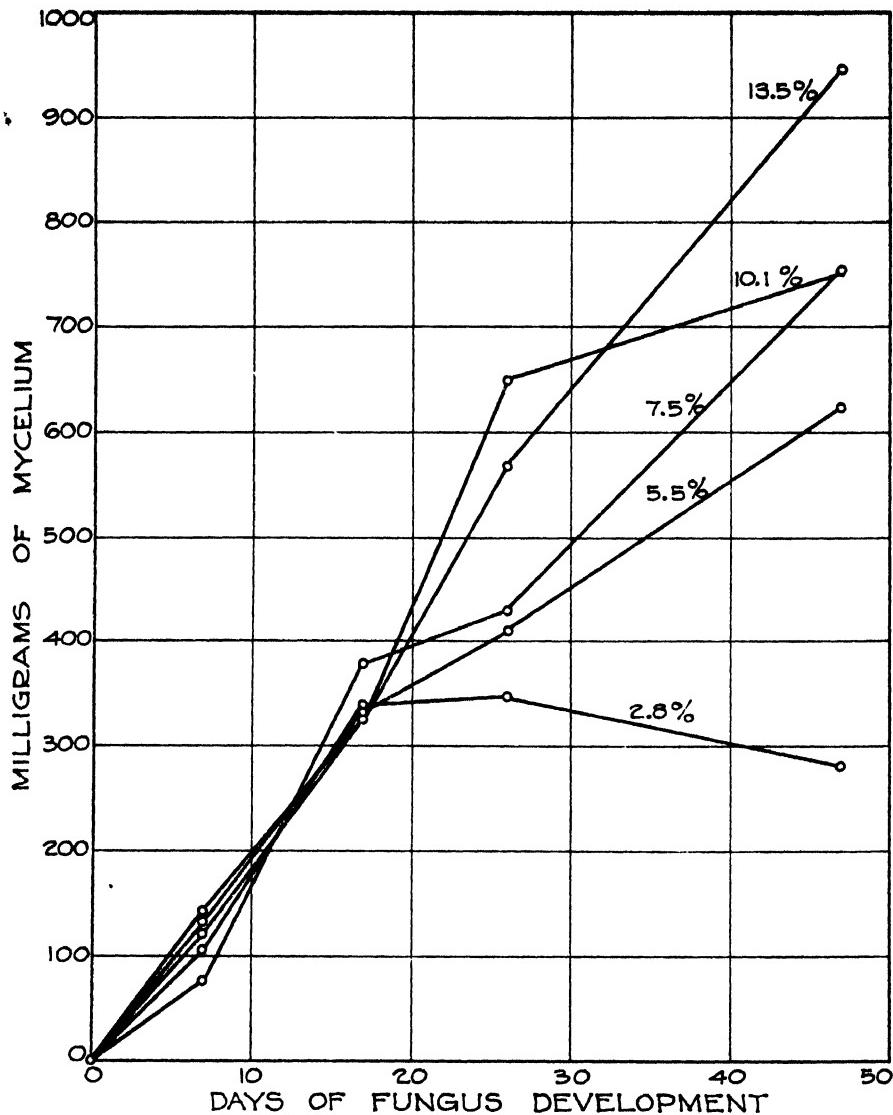


FIG. 2. Weight of *Chaetomium funicola* mycelium produced on Czapek-Dox medium containing different initial concentrations of glucose.

optimal initial pH values appeared, namely, 4.03, 7.20, and 8.15, with the latter value being the lowest of the three. The relation of change of pH of the medium to maximum yield of mycelium suggested the optimum pH for development of the fungus to be near neutrality and slightly on the alkaline side. The shift in pH of all cultures was toward neutrality and the alkaline side. The rate and amount of glucose dissimilation was closely associated with mycelium formation.

## VARYING THE INORGANIC ORTHO-PHOSPHORUS CONCENTRATION OF THE MEDIUM

Cultures containing 10 per cent glucose and various concentrations of  $\text{KH}_2\text{PO}_4$  were prepared. The initial pH values of the medium were adjusted to a uniform level prior to sterilization. The means of analyses of duplicate cultures (Table 3) showed that *C. funicola* rendered the

TABLE 3

EFFECT OF CONCENTRATION OF PHOSPHORUS AS  $\text{KH}_2\text{PO}_4$  IN CZAPEK-DOX MEDIUM ON GLUCOSE DISSIMILATION BY *Chaetomium funicola*

DAYS OF FUNGUS DEVELOPMENT	MILLIGRAMS PHOSPHORUS IN CULTURE PER 35 CC. OF MEDIUM						
	0.0	1.81	7.02	21.13	42.0	104.53	211.3
MILLIGRAMS GLUCOSE PER 1 CC. OF CULTURE MEDIUM							
0	92.7	92.7	91.6	92.0	92.3	92.2	90.1
6	91.2	89.3	80.8	82.6	83.8	87.2	87.0
13	85.4	73.1	73.8	70.4	73.2	76.6	70.4
22	89.1	64.7	60.3	63.9	62.2	59.6	49.4
pH OF CULTURE MEDIUM							
0	4.4	4.4	4.4	4.6	4.7	4.9	4.9
6	6.4	7.4	6.4	6.0	5.6	5.5	5.3
13	6.8	8.0	7.8	6.7	6.4	5.8	5.6
22	6.2	7.4	7.5	6.9	6.5	6.0	5.8
MYCELIUM FORMED, MILLIGRAMS							
6	0.65	60.0	82.3	79.4	87.6	64.5	56.2
13	35.7	287.6	267.8	260.8	233.2	185.4	209.2
22	40.8	400.8	418.0	339.3	355.3	354.4	333.4

medium progressively less acid with development under the different concentrations of  $\text{KH}_2\text{PO}_4$  and that this change was more rapid with the lower concentrations of this salt. A greater increase in mycelial weight accompanied this greater rate of change of pH. Glucose dissimilation, however, did not follow this same trend but rather the inverse of it. More glucose was dissimilated per unit weight of mycelium at the higher than at the lower concentrations of phosphorus. Only slight development of *C. funicola* occurred in the complete absence of any phosphorus additions. The sustained growth obtained was attributed to the presence of phosphorus with the spores, although tests for the presence of inorganic ortho-phosphorus in the water spore suspension were negative. The optimum phosphorus concentration for *C. funicola* activity was approximately 7 mg. of phosphorus as  $\text{KH}_2\text{PO}_4$  per 35 cc. medium, which represented the amount normally contained in Czapek-Dox medium. A reduction of phosphorus to 1.81 mg. per 35 cc. medium did not result in any decreased activity of the fungus.

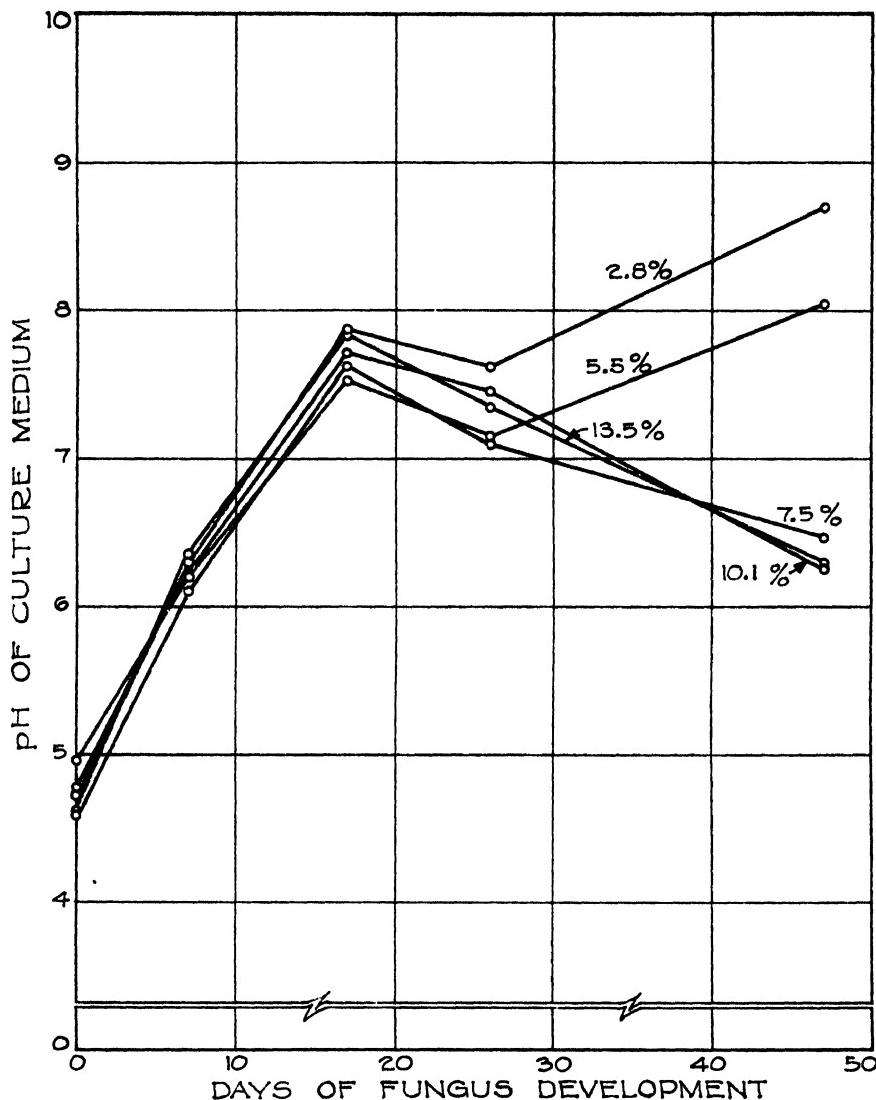


FIG. 3. Changes induced by *Chaetomium funicola* in pH of Czapek-Dox medium containing different initial concentrations of glucose.

#### VARYING THE INORGANIC NITROGEN SOURCE OF THE MEDIUM

The decrease in acidity of the medium in the foregoing experiments might be due either to the production of basic substances such as ammonia as suggested by Dickson (1) or merely to the utilization of nitrate ions from solution. In the absence of any initial formation of organic acids, the effect of the different inorganic nitrogen salts present in the medium would be expressed by a rise or fall in pH, depending on whether such

salts were nitrate or ammonium salts. To test this effect as well as to determine the relative availability of different inorganic nitrogen sources and their relation to the rate of glucose dissimilation, cultures were prepared containing 5 per cent glucose and the different nitrogen sources in amounts equivalent to 17.5 mg. elementary nitrogen (N) per 40 cc. of medium. The nitrogen solutions and the remaining portions of the medium were sterilized separately. Adjustments of the different nitrogen-containing media to a uniform initial pH were made in the non-nitrogen portions prior to sterilization. The mean results obtained by the analysis of duplicate cultures (Table 4) showed that with the exception of  $\text{NaNO}_2$  all other tested inorganic nitrogen sources supported growth of *C. funicola*. The amount of glucose dissimilated paralleled the increase in mycelial weight of the fungus. Sodium nitrite apparently was toxic to *C. funicola* since no growth followed the addition of  $\text{NaNO}_3$  after 3 months development. Ammonium salts supported growth only until the medium was rendered too acid to support further fungus growth. Thus,  $(\text{NH}_4)_2\text{SO}_4$  and  $(\text{NH}_4)\text{H}_2\text{PO}_4$  supported only slight growth because the medium reached a pH of about 3.7 and 3.5, respectively, very early in the development of the fungus, while  $(\text{NH}_4)_2\text{CO}_3$  and  $(\text{NH}_4)_2\text{HPO}_4$  supported greater growth because these salts, being basic, rendered the medium only very slowly acid. Nitrate salts such as  $\text{NaNO}_3$  and  $\text{KNO}_3$  proved most favorable for the development of the fungus because the change in pH shifted toward the alkaline side. Calcium nitrate yielded an unexpected result; only very slight growth of the fungus occurred on this salt during the first 28 days of development, after which a very rapid growth followed. The yield of mycelium obtained on this salt at the termination of the experiment was nearly as great as that obtained on  $\text{NaNO}_3$  and  $\text{KNO}_3$ . On  $\text{NH}_4\text{NO}_3$  even less mycelial yield was obtained than on the foregoing nitrate salts. With this salt the medium did not show any appreciable change in pH during the initial 16 days of fungus development but became more acid following this time, reaching pH 3.64 at the termination of the experiment. The greater availability of the ammonium radical over the nitrate radical followed from a consideration of the pH changes in the medium containing  $\text{NH}_4\text{NO}_3$ . The marked shift in pH to the more acid side in the later period of fungus development indicated the greater utilization of the ammonium ion, while the seemingly constant pH during the early period of development indicated the utilization of both ions to an equal extent. This latter conclusion may be modified by the tendency of the fungus to render the medium more alkaline, as occurred in the nitrogen-free cultures and those containing  $(\text{NH}_4)_2\text{CO}_3$ . A slight preferential utilization of the ammonium ion even in this early period was thus suggested.

#### SUMMARY

1. The effect of some modifications of Czapek-Dox medium on the rate of glucose dissimilation, mycelium formation, and pH changes in the medium by *Chaetomium funicola* Cke. was studied.

TABLE 4  
EFFECT OF SOURCE OF INORGANIC NITROGEN IN CZAPEK-DOK MEDIUM ON GLUCOSE DISASSIMILATION BY *Chaetomium fumicola*

Days of Fungus Development	Milligrams Glucose per 1 cc. of Culture Medium						Mycelium Formed, Milligrams
	(NH <sub>4</sub> ) <sub>2</sub> CO <sub>3</sub> ·H <sub>2</sub> O	(NH <sub>4</sub> ) <sub>2</sub> SO <sub>4</sub>	(NH <sub>4</sub> ) <sub>2</sub> HPO <sub>4</sub>	NH <sub>4</sub> NO <sub>3</sub>	KNO <sub>3</sub>	Ca(NO <sub>3</sub> ) <sub>2</sub>	
pH of Culture Medium							
0	51.5	51.5	51.4	52.5	50.5	52.7	51.5
16	45.3	51.5	41.6	51.5	51.4	43.9	43.4
35	33.6	51.6	37.0	48.9	36.0	41.2	22.8
Mycelium Formed, Milligrams							
0	6.33	4.80	6.95	4.65	4.48	4.65	4.78
16	6.52	3.75	5.02	3.48	4.63	6.98	7.01
35	3.95	3.67	3.67	3.47	3.64	6.38	7.23
Mycelium Formed, Milligrams							
16	149.0	19.4	173.4	32.5	152.3	10.9	168.9
35	273.4	20.0	213.6	43.0	217.8	325.6	403.5
Mycelium Formed, Milligrams							
16	13.6	—	—	—	184.7	445.2	—
35	—	—	—	—	—	—	18.2

2. Changing the glucose concentration of the medium from 2.8 to 13.5 per cent revealed that glucose dissimilation followed a linear path, the rate of dissimilation being higher with the higher concentrations of glucose. The mycelial weights increased uniformly at all concentrations of glucose for approximately the first 17 days of development. Following this time at lower concentrations of glucose (2.8 per cent), the mycelial weights decreased, while at higher concentrations of glucose, the mycelial weights continued to increase. The pH of the media increased progressively to the alkaline side for 17 days of initial fungus development followed by a decrease. This decrease in pH was maintained for the duration of the experiment (47 days) in cultures containing 7.5 per cent and more glucose, while an increase followed in cultures containing less glucose but only after the glucose in the medium was reduced to a low level.

3. Changing the initial pH of the medium revealed that *C. funicola* grew readily over a wide range of pH with no growth at pH 2.12. Growth was reduced more at pH 2.90 than at 8.68. The pH changes in the medium together with the yields of mycelium suggested the optimum pH for development of *C. funicola* to be near neutrality and slightly on the alkaline side.

4. Increasing the concentration of  $\text{KH}_2\text{PO}_4$  in the medium above the normal for the medium (35 mg.  $\text{KH}_2\text{PO}_4$ , or 7.98 mg. P per 35 cc. of medium) resulted in lower mycelial weights and slight increases in amount of glucose dissimilated. Decreasing the concentration of  $\text{KH}_2\text{PO}_4$  to one-fourth of normal did not result in any appreciable decrease in mycelial weights or dissimilated glucose. The rate of change of pH of the medium was greater at the lower concentrations of  $\text{KH}_2\text{PO}_4$  than at the higher.

5. Inorganic nitrogen salts markedly influenced the development of *C. funicola*. Salts such as  $(\text{NH}_4)_2\text{CO}_3$ ,  $\text{NH}_4\text{NO}_3$ ,  $(\text{NH}_4)_2\text{SO}_4$ ,  $(\text{NH}_4)_2\text{HPO}_4$ , and  $(\text{NH}_4)\text{H}_2\text{PO}_4$ , which rendered the medium acid as the nitrogen ions became utilized, were definitely deleterious to the development of the fungus. The rapidity of action of this deleterious influence varied with the salt. Thus,  $(\text{NH}_4)_2\text{HPO}_4$ ,  $(\text{NH}_4)_2\text{CO}_3$ , and  $\text{NH}_4\text{NO}_3$  rendered the medium more slowly acid and hence were less deleterious to the development of *C. funicola* than were  $(\text{NH}_4)_2\text{SO}_4$  and  $(\text{NH}_4)\text{H}_2\text{PO}_4$ . Sodium nitrate,  $\text{KNO}_3$ , and  $\text{Ca}(\text{NO}_3)_2$ , on the other hand, by rendering the medium alkaline, the condition favorable to the development of *C. funicola*, supported the greatest yields of mycelium. The late onset of fungus development on  $\text{Ca}(\text{NO}_3)_2$  remains unexplained. The ammonium ion was utilized preferentially over the nitrate ion. Sodium nitrate did not support growth of the fungus and apparently was toxic.

6. The rate and amount of glucose dissimilation in general was closely associated with mycelium formation.

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# CIRCULATION OF HEMOLYMPH IN THE WINGS OF THE COCK- ROACH, BLATTELLA GERMANICA L.<sup>1</sup>

## III. Circulation in the Articular Membrane: The Significance of this Membrane, the Pteralia, and Wing Folds as Directive and Speed Controlling Mechanisms in Wing Circulation

STEWART CLARE<sup>2</sup> AND OSCAR E. TAUBER

From Insect Physiology Laboratory, Entomology and Economic Zoology Section,  
Iowa Agricultural Experiment Station

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### INTRODUCTION

In an earlier paper, the writers described circulation in normal wings of *Blattella germanica* (2); recently results of experimentally altering the normal circulatory pattern were reported (3). The above studies show that hemolymph in the German cockroach wing circulates in an orderly manner, with larger streams following definite hemolymph channels. Embryological and anatomical accounts of the origin and structure of the wing as a sac-like evagination of the body wall lead one to believe that hemolymph in the appendage is merely an overflow from the hemocoelic volume. However, observational studies (1, 2, 3) in the living insect show orderly currents in hemolymph movement and suggest a definite regulatory mechanism for directing the hemolymph over proper paths in the wing in order to maintain an uninterrupted flow. To prevent conflicting currents in a steady circuit of hemolymph movement throughout the appendage, presumption of such a mechanism is inevitable. Accumulated evidence (1, 2, 3) indicated that these regulatory properties lie in the wing base structures.

The present paper deals with circulation in the wing base (articular membrane); the base's influence on general currents in the channels of the wing proper; and certain circulation velocity controlling mechanisms. It is pertinent to remember that results herein reported are mainly observational, based on direction of hemolymph flow detected by light reflected from moving hemocytes, using the microscope as an aid. No histological section or micro-anatomical studies of directive structures were attempted. A few supplementary gross anatomical observations were made by dissection in connection with experimental sectioning. Detailed description of materials and methods has been given in previous reports (1, 2, 3). Terminology employed is essentially that used earlier and described elsewhere (1, 2). Additional terms are either shown in diagrams or defined in footnotes.

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<sup>2</sup>Now with U.S.D.A. Bureau of Entomology and Plant Quarantine, Chemical Laboratory, White-Fringed Beetle Project, Gulfport, Mississippi.

## RESULTS

Articular membrane<sup>3</sup> circulation is easily detected with a dissecting microscope, by using strong reflected light to discern direction of motion of hemocytes in the hemolymph stream. It is often difficult to observe movement through pigmented and sclerotized pteralia; however, observation of circulation in unpigmented intermediate areas, together with limited data concerning movement below the plates, has provided a better understanding of circulation in the articular membrane. In general, directions of circulation in the membrane of both sets of wings are similar.

### 1. CIRCULATORY PATTERNS IN, AND SIGNIFICANCE OF, THE NORMAL ARTICULAR MEMBRANE

**ARTICULAR MEMBRANE OF TEGMEN (FIG. 1):** Beneath the dorsal surface of the articular membrane, occupied by tegula, humeral plate, first, second, and part of the third axillary plates, and part of medial plates, there is a "mass" movement of hemolymph. This volume extends distad to the bases of longitudinal remigial veins where the mass movement becomes broken up into channel currents supplying the afferent remigial circulation.<sup>4</sup> The area below the pteralia is merely a sinusoidal extension of the hemocoel and has been called the anterior sinus. Hemocoelic hemolymph entering afferent remigial wing circulation gains access to the channels by diversion into the anterior sinus. The observed mass motion appears, therefore, to be a continuation of that in the hemocoel.

Hemocoel<sup>5</sup> circulation immediately below the membrane, between the axillaries and tergal plate margin is caudad (Fig. 1). As circulation proceeds back toward the posterior margin of the wing bearing segment, flow into the articular membrane (jugum) is prevented by fusion of dorsal and ventral membrane surfaces. On reaching the posterior margin of the tergite, the mass of hemocoelic flow is diverted mesad, and can be seen through the transparent intersegmental membrane immediately posterior to the tergal plate margin (Fig. 1).

The above mentioned fusion of surfaces involves that section of the articular membrane known as the jugum (free of axillaries) and a part

<sup>3</sup> Terminology: Articular membrane refers to the entire wing base membrane. The axillary membrane designates that part of the articular membrane containing pteralia (sclerites), while that part containing no sclerites is the jugum.

<sup>4</sup> Distinction between mass movement and channel movement is easily detected in behavior of hemocytes in the two areas. In mass flow, hemocytes move along easily and smoothly; in channels, cells jerk along as they contact the closely-confining channel limits. Differential velocity between hemocytes and hemolymph is greater in the channels than in the larger hemocoelic and anterior sinus spaces where obstruction to hemocytes is less.

<sup>5</sup> It is important to recognize that the depth of this circulation in the hemocoel could not be ascertained. It may be merely a thin sheet, primarily supplying the wing, and different essentially from underlying currents. That the "mass" is confined to the segment, as indicated by its mesad division near the posterior margin, suggests a possible hemocoelic arrangement of tissues aiding in the diversion of hemolymph into the anterior wing sinus. This point should receive further study to ascertain the exact sinus in which this sheet of flow occurs, and the depth to which it extends into the hemocoel.

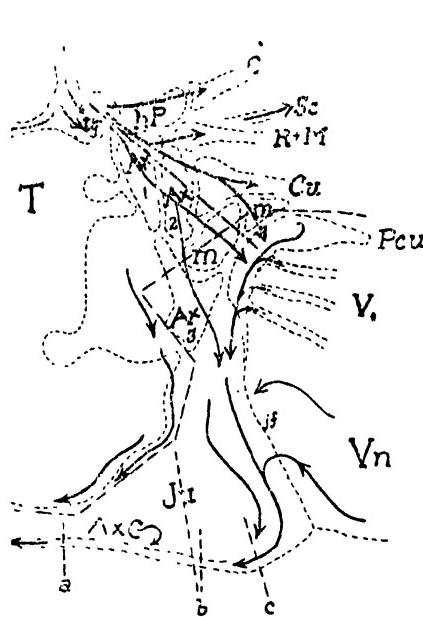


FIG. 1

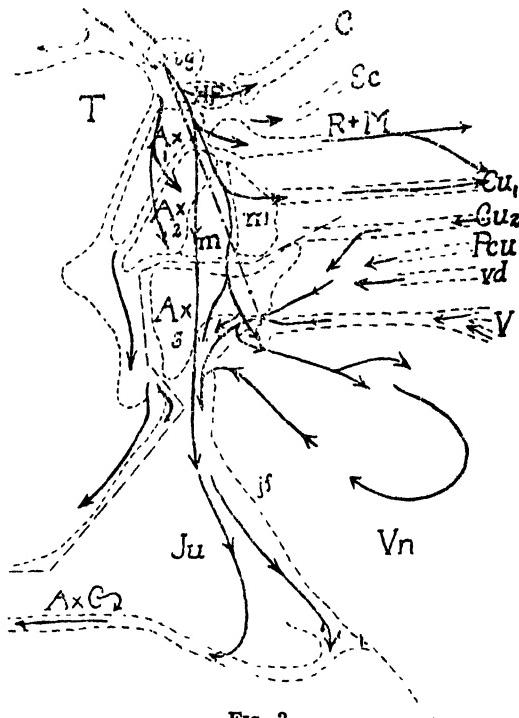


FIG. 2

FIG. 1. Diagrammatic representation of the anatomical arrangement and circulatory pattern in the articular membrane of the tegmen. Structures (pteralia, vein bases—channels, tergite, and jugal fold) are indicated by broken lines (short dashes); basal fold area by dot-dash line; area of fusion of articular membrane surfaces (dorsal and ventral) by broken (long dashes) zig-zag line [anterior sinus is cephalad to line; fused surface containing basal sinus (dilated channel) and jugal channels are caudad to line]. Circulatory pattern and directions of hemolymph movement are shown by arrows.

ABBREVIATIONS: a—indicates a section through the axillary cord (see text); Ax<sub>1</sub> to Ax<sub>3</sub>—first to third axillary sclerites; Ax C—axillary cord; b—section through axillary cord and jugum (see text); c—section through jugal margin (see text); C—costa (abbreviations for all wing veins also indicate the corresponding channels); Cu—cubitus; HP—humeral plate; jf—jugal fold; Ju—jugum; m—medial plate; Pcu—postcubitus; R & M—common radial and medial base; Sc—subcosta; T—tergum or tergite; tg—tegula; V—vannal veins (and channels); Vn—vannal region; vd—vena dividens.

FIG. 2. Diagrammatic representation of the anatomical arrangement and circulatory pattern in the articular membrane of the hind-wing.

of the axillary section including Ax<sub>1</sub> and parts of both medial plates (Fig. 1). Unlike the anterior part (sinus), the fused jugal area contains a system of channels. At the base of the Pcu vein there is also a channel dilation, the basal sinus. Jugal circulation is entirely of efferent hemolymph returning to the general body circulation. The main source is vannal channel hemolymph. Often an additional source is hemocoelic flow passing over one or more short channels in the basal fold area (basal channels). This hemolymph usually enters the basal sinus, and either

joins efferent jugal currents (Fig. 1) or establishes secondary afferent flow in the anterior vannal channels, and returns by vannals near the posterior margin of the tegmen (1, 2, 3). The basal sinus has never been observed to empty into the Pcu channel in the normal tegmen, and it appears that such a connection is either inactive or does not exist. Its absence assists in the complete separation of afferent and efferent currents in the tegmen.

Jugal currents proceed posteriorly to the jugal margin (axillary cord) which they follow toward the general body circulation (Fig. 1). Immediately below the axillary cord hemolymph current within the hemocoel, flow is likewise in a mesad direction, but entirely independent of that coming from the jugal margin. Flow in the hemocoel is somewhat mass movement, while that in the cord is usually more like that in channel motion. Anatomically the axillary cord is a folded continuity of the intersegmental membrane. Observations on hemolymph flow show, however, that the cord channel is functionally discontinuous and completely separate from the hemocoel. It becomes looped (evaginated) and apparently fuses along a line with the segment so that its functional continuity with the hemocoel is lost. All available evidence indicates that it provides a closed vessel for transport of hemolymph from the jugum to the pulsatile organ region.

**ARTICULAR MEMBRANE OF HIND-WING (FIG. 2):** The anterior sinus in the hind-wing is usually somewhat larger than that in the tegmen. In other respects axillary circulation in the two appendages is alike. Circulation in the jugal region is likewise similar; however, there is an essential difference involving the basal sinus. The hind-wing basal sinus is larger. As in the tegmen, it receives hemolymph from the hemocoel over basal channels, but unlike the tegmen it receives a large flow of efferent hemolymph from the remigium via Cu<sub>2</sub>, Pcu, and vd channels. This route is an important outlet for hind-wing remigial streams. As in the tegmen, the remaining hind-wing remigial flow passes into the vannal area and flows efferently through vannal channels to the jugal region. All hemolymph in the remigium of the tegmen passes efferently through vannals (1, 2, 3) and omits entirely the basal sinus. Essentially as in the tegmen, hind-wing basal sinus hemolymph either passes into jugal currents, or establishes secondary afferent currents in certain vannals, and returns to jugal circulation via others.

## 2. EXPERIMENTAL SECTIONING OF ARTICULAR MEMBRANE CHANNELS

Sectioning regions of the articular membrane has aided in establishing its importance as a directive mechanism in wing circulation. Sectioning the axillary cord near the jugal fold will not arrest circulation (Fig. 1, with section indicated at "c"). Establishment of secondary flow in inactive channels carries the stream around or beyond the cuts. Cuts may be made even closer to the tergum without seriously impairing circulation (Fig. 1, indicated at "b" by short dash-line). When a section passes through the cord nearer the segment margin, establishment of secondary

currents is impossible; and, since all hemolymph must pass through the axillary vessel to complete the circuit (1, 2, 3), wing circulation is arrested (Fig. 1, with a cut at "a"). The axillary vessel separation is the most positive and definite way of permanently arresting wing circulation.

Other sections can be performed to alter circulatory patterns, but few arrest circulation. A proper cut extending from the axillary vessel toward the anterior sinus usually stops flow (Fig. 1, indicated at "b" by long broken line).

### 3. SIGNIFICANCE OF PTERALIA AND WING FOLDS

**PTERALIA:** The pteralia give support to the articular membrane, and facilitate entrance of hemocoelic hemolymph into the channels by keeping the membranous surfaces separated. Their function is especially important in the folded wing. Experimental pressure at these sclerotized areas (simulating fused membrane surfaces) retards or arrests circulation. Circulation is likewise affected when a wing is changed from flight position to that of rest (folded), which indicates that the presence of these structures may serve to alter the velocity and volume of flow through the wing. The sclerites also prevent collapse in the basal sinus area, where slight pressure retards or impairs circulation.

**WING FOLDS:** Manipulation of the various wing folds (basal, vannal, and jugal folds) indicates that they have a decided influence on the speed of circulation. When a wing is outstretched in a position simulating flight,<sup>6</sup> the folds are "opened" and circulation is accelerated; on folding to a normal resting position, streaming is arrested. Wing creases offer considerable resistance to flow because of stress put on folded or "doubled back" channels. During this strain on the channels the pteralia assist in preventing complete closure of channels and sinuses in the wing base.

The vannal fold in the tegmen is "vestigial" and plays little part in circulation; however, it extends entirely across the hind-wing. Pressing against a hind-wing along the fold retards vannal currents. Remigial flow above may continue because of efferent flow in the Cu<sub>2</sub>, Pcu, and vd. The influence of the jugal fold on the volume and velocity of circulation is the least difficult to observe. Several channels cross the membrane at this point. When these channels are creased (resting position) or when undue folding pressure is exerted, circulation in the immediate area is arrested. By proper manipulation of this fold it is sometimes possible to arrest circulation in the tegmen. Often stresses on one fold accelerate streaming in other areas, so long as there is an efferent outlet for hemolymph.

Invariably, struggling of specimens attempting to free themselves markedly influences the rate of flow. Sometimes the flow is accelerated; at other times it is retarded, or even arrested. The active muscular

<sup>6</sup>Care must be exerted in outstretching the wing to a natural flight position. Forcible outstretching often retards circulation because of unnatural strains on the channels and other parts.

movements along with the compression and enlargement of the roach's body in such struggling undoubtedly alter the hemocoelic pressure and thus affect circulation in the appendages.

In view of the observation that a wing carefully outstretched to simulate flight position, with all folds and undue stresses "eliminated," possesses an accelerated flow, it is not unlikely that wing circulation may be at a much higher rate during flight. Evidence collected through many observations indicates that speed of hemolymph flow during flight is influenced by the presence of the basal mechanisms and wing folds.

#### 4. SIGNIFICANCE OF REVERSED CIRCULATION

Reversed circulation has a bearing on the definiteness of the hemolymph circuit and the accompanying potential necessary for its movement. Apparently, it sometimes happens that reduced pressure in the hemocoele allows afferent remigial hemolymph to "rush" or flow back into the hemocoele. A reduction in hemocoelic pressure may result from sudden expansion of the body. Generally, reverse in remigial flow is not accompanied by a corresponding reverse in vannal circuit (1, 2). Failure of the vannal efferent currents to reverse may indicate a probable "aspiratory" action of the pulsatile organ in maintaining a steady reduction gradient from anterior sinus to pulsatile organ (1, 2). Normally heart action seems to produce a positive pressure acting synchronously with the negative pressure of the pulsatile organ. The observational evidence suggests that proper distribution of these pressures in maintaining steady and uninterrupted hemolymph flow lies with the articular mechanism.

#### DISCUSSION AND SUMMARY

Study of normal wing circulation (1, 2) has shown that outgoing (afferent) hemolymph flows in channels along the anterior wing margin to the distal apical margin. Afferent flow changes its course in certain definitely outlined wing areas (intermediate circulatory zones), and is forced proximally (efferently) in channels along the posterior wing margin (1, 2). This general and regular flow suggests that a definite circulatory potential is established, whereby the action of hemolymph propelling forces is utilized to maintain a pressure gradient. Preceding successful establishment of this gradient it is necessary that a definite anatomical arrangement of structures be present at the wing base to direct hemolymph through definite channels, and to prevent obstruction of flow by random mingling of opposing currents. The arrangement of the anterior sinus and fusion of the articular membrane about efferent channels evidently provides this type of directive mechanism.

Should the wing be an unmodified sac-like evagination of the body wall, void of hemolymph directing mechanisms, it is not likely that there would be a steady and uninterrupted flow of hemolymph throughout channels of the appendage proper. Equal pressure at all points of a wing base not possessing a directive mechanism would merely result in an inflated, hemolymph-filled appendage, lacking a high and low pressure

area capable of influencing direction and movement. This simple condition can be simulated in the normal wing by sectioning certain vital areas, whereby the potential influence of the propelling organs (3) is destroyed. Failure to observe complete reversal in either normal wing circulation, or under experimental conditions, lends added support to the importance of the axillary structure in directing hemolymph and in distributing properly the force propelling it (1, 2, 3).

#### CONCLUSIONS

1. Two "types" of circulatory movement are found in the articular membrane region; namely, mass and channel movement. The former is confined to larger spaces, such as the adjoining parts of the hemocoele, and the anterior sinus; the latter movement is confined to channels. Behavior of moving hemocytes within channels is different from that of those circulating in the spacious hemocoele. This observation is of considerable assistance in ascertaining the influence of the articular membrane and its contained structures on circulatory direction.
2. Hemocoelic hemolymph enters afferent wing channels through the anterior sinus, a "dilation" of the axillary membrane.
3. Fusion of the posterior area of the articular membrane (jugum) prevents intermingling of hemocoelic and anterior sinus hemolymph with returning efferent wing currents. The fused membrane contains "closed" channels which provide an outlet for efferent hemolymph.
4. Efferent hemolymph flows from the jugum along the axillary cord channel into the pulsatile organ region before returning to the general circulation.
5. Anatomical features of the articular membrane provide an effective mechanism which takes part in allowing an uninterrupted stream of hemolymph to flow through the wing. The mechanism thus acts as an accessory in maintaining a potential or circulatory gradient. Such a gradient is a physical prerequisite for the steady transport of hemolymph through the wing.
6. By cutting through certain vital points along the axillary cord, circulation can be arrested. This operation amounts to the destruction of the circulation potential by the removal of a return pathway. The wing then becomes a sac-like evagination of the body wall, void of a circulatory potential or gradient.
7. The pteralia located in the articular membrane assist in keeping open the circulatory passages through which hemolymph enters the wing.
8. Wing folds have a regulative influence on the velocity of circulation in that, when the folds are closed, much less hemolymph is able to pass across the crease.
9. Evidence indicates that circulation is much more rapid during flight than when at rest. When in a resting position the wings are folded and the creases "obstruct" or retard circulation. When folds are "eliminated" by holding the wing in a simulated flight position, circulation is accelerated.

10. There is only one important difference between the fore- and hind-wing bases. The difference involves the hind-wing basal sinus which, unlike that of the tegmen, receives remigial hemolymph.

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# THE CHILEAN RHOPALIDAE IN THE EDWYN C. REED COLLECTION (HEMIPTERA)

H. M. HARRIS

From the Department of Zoology and Entomology, Iowa State College

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Through the courtesy of Professor Carl J. Drake it has been my privilege to work over the Rhopalidae represented in the Edwyn C. Reed collection, now a part of the Drake hemiptera collection. The study of this material, which formed the basis for Reed's treatment of the group in his "Sinopsis de los Hemípteros de Chile" has contributed much to my understanding of the forms described by Spinola and Blanchard in Gay's Historia de Chile as well as those Chilean forms discussed in the writings of Dallas and Signoret. As indicated below the three names erected by Reed, namely *Harmostes signoreti* Reed, *Harmostes gracilis* Reed, and *Corizus chilensis* Reed, must fall as synonyms of earlier described species.

## *Harmostes raphimerus* (Spinola)

- 1852 *Merocoris raphimerus* Spinola, in Gay, Hist. de Chile, Zool. VII: 164.  
1863 *Harmostes raphimerus* Signoret, Ann. Soc. Ent. Fr., (4)3: 560.  
1900 *Harmostes raphimerus* Reed, Sinop. Hemip. de Chile, p. 51.  
1900 *Harmostes signoreti* Reed, Sinop. Hemip. Chile, p. 52.  
1917 *Harmostes raphimerus* Gibson, Ent. News, 28: 446.  
1941 *Harmostes raphimerus* Torre-Bueno, Bull. Brooklyn Ent. Soc., 36: 84.

Head longer than wide across eyes (37 : 30), the tylus thin and high. Antenniferous tubercle spines slender, acute, diverging anteriorly, the distance from front of eye to apex of spine noticeably greater than length of an eye. Antennae faintly shorter than head, pronotum and scutellum conjoined; segment I from above surpassing tylus by half its own length; II enlarged and somewhat compressed on basal part; proportions, 17 : 25 : 32 : 19 (in some, 17 : 30 : 40 : 20). Bucculae sloping backwards, disappearing in front of a line through front margin of eyes. Rostrum reaching on metasternum, segment I not going beyond hind margin of eyes. Pronotum strongly widened posteriorly, the sides concavely sinuate, the edge sharply reflexed so that there is formed a broad channel within the lateral margin (in some specimens the edge is not reflexed toward the humeri and the lateral channels, therefore, are prominent only along the front half), disc with a distinct median carina, front angles conspicuously produced, humeral angles broad. Scutellum faintly broader than long. Hemelytra with entire clavus and corium except for small elongate area in middle cells of the latter, coriaceous and coarsely punctate; membrane hyaline, sometimes speckled with brown.

LENGTH, 9.8–10.1 mm. WIDTH (across humeri), 3.4 mm.

Several examples of this species are present in the Reed collection and are labeled "*Harmostes raphimerus*." One specimen bears the nota-

tion "Sp. nov. or near *raphimerus*, 1 ant  $\frac{1}{2}$  head." Other specimens, apparently specifically inseparable from those mentioned above, are labelled *signoreti*. One of these, a female, carries the pin label "*Margus* sp. nov." and is presumed to be the type. I have designated this specimen *lectotype*.

It seems evident that Reed based his *signoreti* more on Signoret's description and the inconsistencies between it and Spinola's description than upon any characters he himself was able to discern in the specimens before him. Reed correctly pointed out that Spinola's type specimen must have been a female even though it was described as a male. Spinola's characterization "antennarum articulo primo plus capite longiore" of course is not literally true and puzzled Reed much, and this one character perhaps more than any other led him to create the name *H. signoreti*. I think Spinola really meant that the first antennal segment projected much beyond apex of head. Signoret must have recognized this for he says, "Antennes avec le premier article tres petit, a peine le tiers de la tete." Although the segment is short and stout and appears to be no more than a third as long as the head, when it is subjected to actual measurement under present day techniques it proves to be decidedly more than a third as long as the head. Antennal II is short, distinctly swollen and somewhat compressed at the base; in some examples it is not as long as distance from apex of tylus to a line across base of eyes, whereas in others it is about as long as from apex of tylus to the ocelli.

Gibson has placed *Harmostes montivagus* Distant as a synonym of *raphimerus* Spinola, but size alone would seem to preclude this. Torre-Bueno has followed Reed in considering the discrepancies between Spinola's and Signoret's descriptions and considers that two species are involved. However, it is presumed that Signoret had Spinola's types available for study. It is true also that there is some variation in the antennal segments, both in relative lengths of the segments and in the degree of enlargement of the base of the second segment, and that the flattened nature of this segment is not evident when the antennae are viewed from certain angles. In view of all these things, I am forced to consider *signoreti* Reed an outright synonym of *raphimerus* (Spinola).

#### *Harmostes minor* (Spinola)

- 1852 *Merocoris minor* Spinola, in Gay, Hist. de Chile, Zool., VII: 165.
- 1852 *Harmostes chilensis* Dallas, List. Hemip. II: 521.
- 1863 *Harmostes minor* Signoret, Ann. Ent. Soc. Fr., (4) 3: 561.
- 1870 *Harmostes minor* Stål, Enum. Hemip., I: 220.
- 1900 *Harmostes chilensis* Reed, Sinop. Hemip. Chile, p. 52.
- 1917 *Harmostes minor* Gibson, Ent. News, 28: 448.
- 1941 *Harmostes chilensis* Torre-Bueno, Bull. Brooklyn Ent. Soc., 36: 85.

Head subequally as long as broad across eyes (25 : 24). Antennae short, not as long as head, pronotum and scutellum conjoined; segment I as seen from above projecting beyond tylus by half its length; proportions, 17 : 19 : 20 : 15. Spines of antenniferous tubercles fairly prominent; from above slender, directed straight forward. Bucculae tapering

posteriorly, extending a little beyond a line through front margin of eyes. Rostrum reaching to a point between middle coxae; basal joint not quite attaining base of head. Pronotum trapezoidal, the sides almost straight, somewhat expanded and reflexed so that there is a broad sulcus within for the full length, the edge itself minutely granulate; front angles slightly produced, humeral angles rather broad; distance across humeri not greater than distance across base of hemelytra. Scutellum subequally as long as broad, its margins raised, the apex broadly rounded. Clavus and exocorium opaque and coarsely punctate, mesocorium hyaline, impunctate except for a marginal row. Membrane hyaline, without specks or dark streaks. Apex of last genital segment of male broadly excised beneath; the clasper small, its tip dark and recurved.

LENGTH, 6.1–7.4 mm. WIDTH (across humeri) 2.0–2.5 mm.

There are several specimens in the collection, and one bears the label "*Harmostes minor?*" These examples agree well with Dallas' description of *chilensis* and also fairly well with the description of *minor* except for size, and, of course, the hind femora are armed. Just what Spinola meant when he said "pedibus inermibus" is quite puzzling, and if he were correct in so characterizing his specimens the species must be referred to some other genus. It is presumed, however, that Signoret saw Spinola's types, and he referred them to *Harmostes* and placed *chilensis* Dallas as a synonym. I have found no evidence to support Reed's statement that Gay's Volume 7 did not appear until 1853 or 1854 and have, therefore, followed Signoret and Stal in giving precedence to Spinola's name.

The species is related to *Harmostes procerus* Berg.

#### *Harmostes marmoratus* Blanchard

- 1852 *Merocoris marmoratus* Blanchard, in Gay, Hist. de Chile, Zool. 7: 166.
- 1863 *Harmostes marmoratus* Signoret, Ann. Ent. Soc. Fr., (4) 3: 561.
- 1900 *Harmostes marmoratus* Reed, Sinop. Hemip. Chile, p. 53.
- 1900 *Harmostes marmoratus* Berg, Anal. Mus. Nac. Buenos Aires, 7: 85.
- 1917 *Harmostes marmoratus* Gibson, Ent. News, 28: 446.
- 1841 *Harmostes marmoratus* Torre-Bueno, Bull. Brooklyn Ent. Soc., 36: 86.

Small, slender; greenish yellow, usually with a series of brownish spots along expanded margin of pronotum and hemelytra, sometimes strongly roseate or the pronotum and clavus in part strongly embrowned. Head subequally as broad as long (22 : 23), the anteocular part to apex of antenniferous tubercle as seen from the side barely as long as an eye. Antennae subequally as long as head, pronotum and scutellum combined; segment I surpassing apex of tylus by less than half its own length; proportions, 12 : 17 : 19 : 15. Antenniferous tubercles from above slender, not noticeably divaricating, from the side rather stout and rectangular with dorsal edge slightly produced. Rostrum not attaining hind coxae, segment I barely reaching beyond a point opposite middle of eye. Bucculae sloping posteriorly, terminating opposite front of eye. Pronotum distinctly raised and widened behind front lobe, the median length just one-half width across humeri, the lateral margins narrowly

expanded and reflexed; the anterior angles short, acute; the humeral angles rounded, the distance across them not greater than width across base of hemelytra. Scutellum as long as broad, its margins raised. Hemelytra with entire clavus and corium, except for distal half of middle basal cell, strongly punctate. Membrane hyaline, immaculate. Genital capsule of male with its posterior margin roundly convexly produced at the middle between the projecting claspers. Claspers recurved apically as in *H. minor*, but smaller and with different apex.

LENGTH, 5.5–6.1 mm. WIDTH (across humeri), 1.6–2.0 mm.

This form is closely related to *H. minor*. It is, however, smaller and slenderer, the lateral pronotal margins are less broadly expanded and more sharply reflexed, the pronotum is more suddenly widened so that its lateral edge is distinctly concave (especially noticeable in the male), the tylus extends beyond middle of first antennal segment, the inner and distal cells of corium are punctate, and the male genital characters are distinctive. As in *rhabphimerus* and *minor* there is much variation in color, the pale, more or less immaculate examples being easily confused with *H. minor*. Judging by the specimens, I have seen Gibson was in error in saying the membrane is spotted with fuscous. Torre-Bueno has followed Gibson in this even though there is no mention of such in the descriptions of Blanchard, Signoret, and Reed. *Harmostes corazonus* Distant, which Gibson placed as a synonym of *marmoratus*, is described as having a speckled membrane and for the time being must be considered as a valid species.

*Xenogenus gracilis* (Reed)

- 1900 *Harmostes gracilis* Reed, Sinop. Hemip. Chile, p. 53.  
 1941 *Harmostes gracilis* Torre-Bueno, Bull. Brooklyn Ent. Soc., 36: 84.

In the Reed collection under the name *Harmostes gracilis* there are several examples of a species that belong to *Xenogenus* Berg. That Reed was in doubt about the generic position of the species is evident by the fact that one of his specimens carries the label "nov. gen. et sp. near *Harmostes*." This specimen is a male in good condition and I have designated it lectotype.

*Xenogenus gracilis* (Reed) is close to *X. picturatum* Berg and *X. extensum* Distant, and a study of a long series of examples from many localities will be necessary for an understanding of their true relationships. In Reed's Chilean examples the male clasper is swollen at the middle and constricted before the apex as in examples of *X. extensum* from Arizona but is decidedly larger.

*Liorhysuss hyalinus* (Fabr.)

- 1794 *Lygaeus hyalinus* Fabricius, Ent. Syst., 4: 168.  
 1835 *Corizus gracilis* Herrich-Schaeffer, in Panzer, Fauna Germanica, p. 127.  
 1842 *Corizus truncatus* Rambaur, Fauna Andal., 2: 144.  
 1852 *Merocoris lineatoventralis* Spinola, in Gay, Hist. de Chile 7: 168.  
 1852 *Merocoris maculiventris* Spinola, Gay, Hist. Chile, 7: 170.  
 1852 *Merocoris microtomus* Spinola, Gay, Hist. Chile, 7: 171.  
 1852 *Merocoris rubescens* Blanchard, in Gay, Hist. de Chile, 7: 173.

- 1859 *Corizus gracilis* Signoret, Ann. Soc. Ent. Fr., (3) 7:88.  
 1859 *Corizus quadrilineatus* Signoret, Ann. Soc. Ent. Fr., 7:90.  
 1870 *Liorhyssus hyalinus* Stal, Enum. Hemip., 1: 222.  
 1900 *Corizus gracilis* Reed, Sinop. Hemip. Chile, pp. 54, 55.  
 1900 *Corizus chilensis* Reed, Sinop. Hemip. Chile, p. 55.  
 1900 *Corizus maculiventris* Reed, Sinop. Hemip. Chile, p. 55.  
 1900 *Corizus microtomus* Reed, Sinop. Hemip. Chile, p. 56.  
 1900 *Corizus lineatoventris* Reed, Sinop. Hemip. Chile, p. 56.  
 1900 *Corizus quadrilineatus* Reed, Sinop. Hemip. Chile, p. 57.  
 1900 *Corizus gracilis* Berg, Anal. Mus. Nac. B. Aires, VII: 85.

In the Reed collection there are a number of specimens of *Liorhyssus hyalinus* (Fabricius) representing many of its color variations. These were segregated by Reed and stand under the name labels attached by him as indicated below.

*Corizus gracilis* is so labeled. In the literature this name has already been synonymized with *hyalinus* Fabricius.

*Corizus chilensis* is represented by three specimens each bearing the label "rubescens." One specimen is labeled "Salta, 19-11-87, rubescens = chilensis." These agree fairly well with Reed's and Blanchard's characterizations and seem to be specifically inseparable from *hyalinus*. The names *Merocoris rubescens* Blanchard and *Corizus chilensis* Reed must therefore be considered as synonyms of *hyalinus* Fabr.

*Corizus maculiventris* (Spinola) is represented by four specimens which are specifically identical with those labeled *rubescens* though they are not so strongly marked above with red, and the ventral black patch is larger, extending well back on the venter. In general these Chilean specimens are a little larger and stouter than specimens of *L. hyalinus* from North America and Europe. The name *maculiventris* may well be retained as a varietal name when the color forms of this almost cosmopolitan species are worked out.

*Merocoris microtomus* Spinola is only a pale form of *L. hyalinus*. The specimens in the Reed collection agree well with the descriptions of both Spinola and Reed.

*Merocoris lineatoventris* Spinola and *Corizus quadrilineatus* Signoret constitute a color variety of *hyalinus* in which the venter is marked with four more or less distinct longitudinal dark stripes.

#### *Anhyssus tricostatus* (Spinola)

- 1852 *Merocoris tricostatus* Spinola, in Gay, Hist. de Chile, 7:172.  
 1859 *Corizus annulatus* Signoret, Ann. Soc. Ent. Fr., (3) 7:98.  
 1859 *Corizus tricostatus* Signoret, Ann. Soc. Ent. Fr., (3) 7:104.  
 1900 *Corizus tricostatus* Reed, Sinop. Hemip. Chile, p. 57.  
 1900 *Corizus annulatus* Reed, Sinop. Hemip. Chile, pp. 54, 57.

In the Reed collection there are specimens of this form labeled "annulatus." Reed himself, however, considered Signoret's species as probably identical with Spinola's, an opinion in which I concur. The species is recognized by its small size, the long, rather upright clothing hairs, the speckled legs, antennae and wing veins, the maculate connexivum, the short antennae, and the characteristic claspers of the male. The apical antennal segment is pale at base and apex.

*Niesthrea fenestratus* Signoret

- 1859 *Corizus fenestratus* Signoret, Ann. Soc. Ent. Fr., (3) 7:93.  
1900 *Corizus fenestratus* Reed, Sinop. Hemip. Chile, p. 56.

There are no specimens in the Reed collection that can be placed as *fenestratus*. A careful study of the description leaves me with the impression that the species probably should be referred to *Niesthrea*.

# FLUORESCENT BACTERIA IN DAIRY PRODUCTS<sup>1</sup>

E. R. GARRISON AND B. W. HAMMER

From the Department of Dairy Industry, Iowa State College

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Fluorescent bacterial colonies occasionally are noted on agar plates prepared from dairy products, particularly when certain media are used and the plates are incubated at temperatures below 37°C. Addition of the responsible organisms to milk, cream, and certain derivatives of them commonly results in objectionable but variable changes.

Direct plating can detect fluorescent bacteria only when they are so abundant in a product that they are not diluted out in plates showing a good colony distribution; accordingly, it gives an inadequate idea of the distribution of the organisms. The work herein reported deals (a) with the distribution of the fluorescent bacteria in dairy products as shown by enrichment procedures and (b) with the action of the organisms on milk and butter.

## HISTORICAL

Various investigators have pointed out the conspicuous action on milk and butter of fluorescent bacteria isolated from different materials.

From a sample of cheesy butter Krueger (7) obtained various organisms, one of which was stated to be *Bacillus fluorescens non-liquefaciens*, although it was described as a non-motile rod that produced terminal spores and grew both aerobically and anaerobically. At 16° to 18°C., the optimum temperature, a foul fermentation was quickly produced in sterile milk; the liquid developed a yellow color with a green fluorescence and finally became slimy; the reaction gradually turned acid and after 10 days there was a penetrating odor of trimethylamine; ammonia and hydrogen sulfide also were formed. Butyric and formic acids were produced from triglycerides; it appeared that the higher molecular weight fatty acids were decomposed to these two acids during fat hydrolysis.

Lafar (9) isolated an organism that produced rancidity in butter when inoculated into the cream before churning and named it *Bacillus butyri fluorescens*; however, Reinmann (18) found this organism to be *Bacillus fluorescens liquefaciens*.

Reinmann (18) inoculated *Bacillus fluorescens liquefaciens* into butter churned from sterile cream. The butter had a fresh, desirable flavor when first prepared but developed a strong undesirable odor in a few days and was inedible in 2 or 3 weeks, although not rancid.

Schreiber (20) noted that *Bacillus fluorescens liquefaciens* was the organism most frequently encountered on agar plates prepared from sur-

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faces of cylinders of butter that had been buried in the soil for various periods. Five cultures of the organism, isolated from soil and water, all decomposed cylinders of sterile butter at room temperature when inoculated on the surfaces. Cultures that were lipolytic when first isolated lost their ability to attack fat after being carried on gelatin for 1 year and 9 months. Schreiber stated that in the presence of nutritive material and oxygen the organism breaks down fat and destroys the free fatty acids after they react with calcium carbonate.

Laxa (10) found that when *Bacillus fluorescens liquefaciens* had grown in butter for 1 month the total acid number of the butterfat was 197.4 and the volatile fatty acid number was 0.94; the control fat had a total acid number of 12.2 and a volatile fatty acid number of 5.05. The data were considered to indicate that the organism splits primarily glycerides of the non-volatile fatty acids. Laxa believed that the organism was unable to decompose the higher fatty acids to butyric and formic acids, as suggested by Krueger (7).

Several microorganisms were investigated by Orla-Jensen (16) from the standpoint of their importance in the development of rancidity in butter under commercial and laboratory conditions. *Bacillus fluorescens liquefaciens* was always found in fresh butter, while *Bacillus fluorescens non-liquefaciens* was encountered only occasionally; rancid butter often contained the former organism but not the latter. *Bacillus fluorescens liquefaciens* was one of the two predominating liquefying bacteria in the surface layers of sweet cream butter held at room temperature for 3 days but was present only in small numbers in sour cream butter similarly held. It did not grow in the interior of butter and died off in the surface layers when the volatile fatty acid number reached about 3.5. Butter made from sterile sweet cream had an objectionable taste and a butyric acid-like odor after 1 week and was completely inedible after 2 months. The organism supposedly decomposed butterfat uniformly but utilized the non-volatile fatty acids in preference to the volatile ones. Milk-souring bacteria did not prevent the growth of *Bacillus fluorescens liquefaciens* in butter but, when sufficient acid had developed, hydrolysis of the fat was believed to be retarded. The addition of 2.9 per cent salt (21.6% brine) to butter prevented growth and fat hydrolysis by the organism. Since *Bacillus fluorescens liquefaciens* is widespread in water, it was believed to be introduced into butter from this source. Pasteurization of cream at 85°C. destroyed all organisms injurious to butter.

Kruyff (8) isolated nine species of fat-splitting bacteria from soil, sewage, water, old butter, and animal feces, all of which grew at 37°C. The only one studied to any extent was identified as *Bacillus fluorescens liquefaciens*.

Wolff (25) obtained *Bacterium fluorescens* from part of the milk samples examined but only after they had been held at a low temperature (5° to 7°C.) for several days (2 to 7). Under these conditions fluorescent bacteria comprised from 22 to 42 per cent of the total flora of certain samples. When the samples were held at 20°C., fluorescent colonies were encountered only occasionally on plates poured with the milk.

According to Barthel (1), *Pseudomonas fluorescens* splits fat to cause rancidity but is incapable of attacking glycerol.

Luxwolda (14) grew *Streptococcus lactis* and *Bacterium fluorescens liquefaciens* together in milk and found that at 10°, 13°, or 15°C. both species appeared to profit by the association. After 6 days at 15°C., the milk was sour and coagulated and contained 1,700,000,000 *Streptococcus lactis* and 4,000,000 *Bacterium fluorescens liquefaciens* organisms per ml. Since *Bacterium fluorescens liquefaciens* lived in a sour medium, it appeared that the milk-souring bacteria produce something besides acid that hinders the growth of the fluorescent organisms in sour milk. *Bacterium fluorescens liquefaciens* produced a rennin coagulation and then peptonized the milk. At 3° to 5°C. the odor and taste of milk inoculated with the organism remained completely normal up to 19 days, even though it contained over 300,000,000 bacteria per ml., but after 20 days the milk was bitter and coagulated with alcohol.

Gubitz (4) isolated several cultures of *Bacterium fluorescens* and related organisms, which included *Bacterium punctatum* and *Bacterium putidum*, from milk, butter, soil, water (from various sources), and plants and studied their growth temperatures. The optimum temperature of the organisms was under 30°C., and they were found to be one of the important types that grow at 0°C. Cultures of *Bacterium fluorescens* with a maximum growth temperature of about 35°C. were designated warm forms, while those with a maximum growth temperature of about 27° to 30°C. were termed cold forms. The maximum and optimum temperatures of these organisms were reduced about 5°C. from the original by growth in liquid medium for several generations at 0°C. The physiological properties, particularly the lipolytic and proteolytic characteristics, were not altered by holding at 0°C. for considerable periods. Growing the warm forms for 24 transfers at 30°C. raised the optimum and maximum growth temperatures about 5°C. Two types of colonies—(a) bluish, lobated, irregular-shaped, and spreading and (b) round, raised, discoid, smooth, and glassy-appearing—were produced by the fluorescent organisms. *Bacterium fluorescens* developed an intense bitter taste in milk in 2 days at 18°C. and later peptonized the milk; hydrolyzed fat at 12° and 18°C.; failed to produce hydrogen sulfide; gave only weak growth in media containing 5 per cent sodium chloride; grew at a pH as low as 5.4 to 5.8; and was destroyed at 63° to 64°C. for 30 minutes.

Shutt (21) found that contaminated water used in washing butter was responsible for an unclean, putrefactive flavor which developed on the surface of the butter during holding. The off flavor appeared chiefly during the spring and summer months and was particularly common after periods of heavy rains. It was noted in butter from creameries having good water supplies. In creameries having difficulty with off-flavored butter the water supplies contained large numbers of putrefactive bacteria, chief of which was *Pseudomonas fluorescens*. Sterile butter inoculated with this organism developed the typical surface flavor in 28 days at 25°C. Heating the water to 87.8°C. for 10 minutes was necessary to destroy the organism. The trouble disappeared when the water was

treated or when pure water was substituted for a contaminated supply. Neutralizing the cream to not less than 0.35 per cent acid was beneficial in avoiding the defect, since the organism grew but feebly at pH 6.6. Surface taint occurred only in sweet cream or neutralized cream butter and never developed in sour cream butter.

Löhnis (11) reported that *Bacterium fluorescens* and closely related forms, which regularly are present in water, play an important role in the development of rancidity in butter stored with access to air. Since the organisms grow at low temperatures and decompose fat and protein, they also were detrimental to the flavor of milk.

Newman (15) examined three samples of milk with a bitter flavor and found that they contained mainly *Pseudomonas* organisms. The bacteria grew well at 4°C. and produced a fluorescent blue-green, yellow, or red pigment on agar plates. Colonies picked into sterile milk produced a strong quinine-like bitterness in the milk in 24 to 48 hours at room temperature. A different species of *Pseudomonas* was isolated from each sample of milk. One of the organisms corresponded to *Pseudomonas ovalis*.

Orla-Jensen (17) reported that *Bacterium fluorescens liquefaciens* frequently played an important part in the development of rancidity in butter. *Bacterium pyocyanum*, however, grew so slowly at ordinary temperatures that it did not spoil butter under normal conditions. Since *Bacterium fluorescens liquefaciens* often is added to butter by wash water or ice, Orla-Jensen advised pasteurizing wash water or treating it with chlorine. He stated that this organism produces a turnip, tallow, and sometimes a soap flavor in milk; turnip flavor frequently was noted in milk that had been held at a low temperature.

Rumment (19) stated that numerous investigators have demonstrated *Bacterium fluorescens liquefaciens* was a usual inhabitant of polluted water and caused rancidity in butter. The organism was used in experimental work to determine the number of microorganisms that passed from the wash water into the butter and also the effect of these organisms on the keeping quality of butter. Sweet cream butter absorbed more organisms from the wash water than sour cream butter; the firmer the consistency and the larger the butter granules the fewer were the bacteria traceable to the wash water; and in sweet cream butter (pH 6.8) the organism increased rapidly and decomposed the fat intensely at higher temperatures but only slightly at lower temperatures, while in sour cream butter (pH 4.2 to 4.3) it did not increase, and the fat remained unchanged in the cold but developed an unclean, tallow flavor at higher temperatures. The fatty acids formed in butter were found to have a germicidal effect on the organism.

Virtanen (24) listed the more common defects produced in butter by bacteria as fermented, cheesy, putrid, and "rank." The enzymes causing the defects were stated to be formed by proteolytic water bacteria of the *Pseudomonas fluorescens* and *Pseudomonas punctatum* groups. These bacteria were not easily destroyed by heat but were inhibited by

the acidity of sour cream butter and by salt. It was noted that they usually do not cause defects when the water supply is uncontaminated and the milk is delivered daily; when the milk or cream is 2 or 3 days old, difficulty may arise even though the water supply is pure. The enzymes of these bacteria were not destroyed during pasteurization of the cream and sometimes caused defects in butter in the absence of living bacteria. The catalase test for butter was recommended as a test for the presence of proteolytic bacteria, although a negative test was no assurance that spoilage would not occur.

Henneberg (6) reported that protein decomposition without acid production is typical of the fluorescent group of bacteria, even though many cultures can ferment lactose and dextrose. Protein decomposition, however, can be largely inhibited by the presence of sugar, as is illustrated by the fact that gelatin-liquefying cultures sometimes do not liquefy gelatin when sugar is added. Air favors an alkaline development, while lack of air favors an acid fermentation. Of two gram-negative, gelatin-liquefying, fluorescent organisms, one produced a bitter putrefaction in milk and the other a soap flavor; both strongly hydrolyzed fat; both produced a putrid odor and ammonia in peptone broth; both grew well at 6° to 8°C.; and 7.5 per cent salt was endured by one organism but only 5 per cent by the other. It was stated that *Bacterium fluorescens* and other alkali-forming bacteria are important in decomposing the fat and protein of butter, but low temperatures, acid, and salt are preserving factors.

The ability of *Pseudomonas fluorescens* to split fat was confirmed by Berry (2).

Stark and Scheib (22) studied 486 cultures of lipolytic and caseolytic bacteria isolated from butter prepared and held under known conditions. Included were 40 cultures that resembled *Pseudomonas aeruginosa* in physiological properties; 30 of them produced a blue-green pigment, soluble in water, that turned dark brown with age and became red in the presence of acid. The remaining 10 cultures were identical with the 30 in all respects except that they produced a yellow pigment, slightly soluble in water, the color of which did not change in the presence of acid. This group was assumed to be a variant of *Pseudomonas aeruginosa*.

Storck (23) noted that alkali-forming bacteria constitute an important part of the milk flora during the winter months when the population of acid-forming bacteria is low. The *Bacterium fluorescens* group was one of the alkali-forming types present in raw milk, and three strains were isolated. These hydrolyzed fat and liquefied gelatin; two cultures coagulated milk before digestion, while the other culture digested milk without coagulation. Pasteurization at 63°C. for 30 minutes destroyed all the alkali-producing bacteria in milk except the spore-forming group.

Hansen (5) added 0.05 per cent of a milk culture of *Pseudomonas fluorescens* to milk used for cheese making and found that it did not significantly affect the flavor score or the nitrogenous decomposition in the cheese.

## METHODS

### DETECTION OF FLUORESCENT BACTERIA ON PLATES

Materials were examined for fluorescent bacteria by plating on beef infusion agar (pH 7.0 to 7.2) and incubating the plates 72 hours at 20° to 30°C., these conditions being favorable for production of the fluorescent pigment. In only a portion of the trials were fluorescent colonies on the plates evident from general observations, and plates regularly were examined under a relatively pure ultra-violet light in a dark room. Many of the fluorescent colonies were picked for purification and study.

### ENRICHMENT PROCEDURES

Attempts to isolate fluorescent bacteria by direct plating of fresh milk and cream and products made from them seldom were successful because of the limited numbers of the organisms in such materials, and enrichment procedures were employed. With milk and cream these consisted of holding at 5° to 7°C. for some days and then plating.

Ice cream was allowed to melt at room temperature and plated soon after melting and again after holding at 5° to 7°C. for 5 days; with some samples, 11 ml. of the melted ice cream also was added to 99 ml. of sterile water and held at 5° to 7°C. for 5 days before plating.

Butter was plated directly in dilutions from 1:10 to 1:100,000. The enrichment procedure consisted of adding about 5 ml. of melted butter to a tube of litmus milk, shaking the tube, and plating after holding at 5° to 7°C. for 3 to 6 days.

Water samples were plated when received and also after holding at 5° to 7°C. for several days with about 5 per cent sterile milk added; the latter procedure was much the more effective. Such miscellaneous substances as feed, manure, and soil were examined by placing small portions in bottles of sterile water and plating after holding at 5° to 7°C. for a few days.

It is probable that enrichment attempts were not always successful and that fluorescent organisms sometimes were overgrown. Also, it is probable that even with enrichment the organisms were not obtained from all samples containing them because they could not always be detected on plates. Overcrowding of plates with non-fluorescent types tended to obscure fluorescent colonies since sometimes they were not observed on badly crowded plates but were noted on plates poured with higher dilutions of the sample. Deep subsurface colonies were not fluorescent because of an insufficient oxygen supply for pigment production; thin layers of agar in the plates overcame this difficulty to some extent.

## EXPERIMENTAL

### PRESENCE OF FLUORESCENT BACTERIA IN DAIRY PRODUCTS

**MILK.** Of 274 lots of milk delivered to plants in Iowa and Missouri, 178 (65.0%) yielded fluorescent organisms. Plates poured with some of the samples after enrichment contained mostly fluorescent organisms; the off flavors in such samples were stale, bitter, nutty, and rancid. Thirty-

five bottles of raw milk from the same areas yielded the organisms in 18 (51.4%) instances.

Fluorescent organisms also were isolated from 11 (44.0%) of 25 bottles of pasteurized milk from various dairies in the two states. After enrichment one sample contained mainly fluorescent organisms. Since the fluorescent bacteria are not heat resistant, they presumably were added from the equipment, etc., after pasteurization.

In connection with an investigation on mastitis, 580 samples of milk drawn aseptically from the individual quarters of 145 cows in four dairy herds were plated directly on beef infusion agar and the plates incubated 48 hours at 37°C. Fluorescent colonies were obtained from one or more quarters of four of the cows in numbers ranging from 500 to 4,500 per ml. The organisms isolated were identified as *Ps. aeruginosa*.

**RAW SWEET CREAM.** Of 149 lots of sweet cream delivered to plants in Iowa and Missouri, 87 (58.4%) yielded fluorescent organisms. After enrichment the organisms greatly predominated in several of the samples; the off flavors in such samples were unclean, bitter, cheesy, putrid, and rancid.

**RAW SOUR CREAM.** Fluorescent bacteria were obtained from 115 (77.7%) of 148 lots of sour cream, most of which were collected in Missouri. Of 104 lots examined earlier without enrichment, only 5 (4.8%) yielded the organisms. The acidities of the samples were not determined but varied from slightly sour to high acid.

**ICE CREAM.** Thirty-eight lots of ice cream from commercial plants and counter freezers in several states yielded fluorescent bacteria in 7 (18.4%) instances. The comparatively high sugar contents may have prevented any relative increases in the fluorescent organisms during enrichment.

**FRESH, SWEET CREAM BUTTER.** One hundred and thirteen samples of fresh, sweet cream butter were obtained from Iowa creameries at intervals during January to May, inclusive. Most of the samples were unsalted, and these were made both with and without butter culture; all were of high quality and except in a few instances scored 38 on flavor. Fluorescent organisms were isolated from 39 (34.5%) of the samples.

**OTHER BUTTER.** Of 72 samples of butter of miscellaneous types from Iowa, Missouri, Nebraska, and Oklahoma, 20 (27.8%) yielded fluorescent bacteria. Seven of the samples were farm butter and 2 (28.6%) contained fluorescent organisms. There were 24 samples of stored, unsalted, sweet cream butter that showed certain defects; of these 7 (29.2%) yielded fluorescent species. The remaining 41 samples were from sour cream; 22 were unsalted and some were prepared with use of butter culture. Eleven (26.8%) of the samples yielded fluorescent organisms.

#### PRESENCE OF FLUORESCENT BACTERIA IN MISCELLANEOUS MATERIALS

Fluorescent bacteria were found in 47 (95.9%) of 49 samples of water collected from pasture ponds, roadside pools, streams, rivers, farm

wells, and the roof of a building; also in 9 (75.0%) of 12 municipal water supplies. They were isolated from 15 (93.8%) of 16 samples of miscellaneous materials from a dairy barn and surroundings which included dust from the air and floor, cow feces, bedding, soil, alfalfa hay, beet pulp, cotton seed meal, linseed meal, ground corn, mixed grain, wheat bran, green wheat, green barley, and leaves of crab and hawthorne trees; one sample of each of the materials was examined, and all but one, the sample of linseed meal, gave positive findings.

#### GENERAL CHARACTERS OF FLUORESCENT BACTERIA ISOLATED

Of the cultures of fluorescent bacteria obtained from dairy products and miscellaneous sources, 496 were studied in some detail. They regularly were gram-negative, non-spore-forming rods, with polar flagellation, which grew well on the common media. In broth all the cultures grew at 7°C. and most of them grew at 3°C. All grew at 32°C.; a considerable number failed to grow at 37°C., although a few grew at 45°C.

Many of the cultures could not be identified on the basis of published descriptions.<sup>2</sup> All of them were placed in the genus *Pseudomonas*, although it is possible some of them would have been placed in the genus *Phytomonas* if pathogenicity for plants had been considered. The cultures which could be identified with considerable certainty showed minor variations within a species which indicates that this point must be considered in the preparation of descriptions of the organisms.

#### ACTION OF FLUORESCENT BACTERIA ON MILK

The changes produced in tubes of litmus milk at 21°C. by the 496 cultures of fluorescent bacteria were of six general types as follows:

- a. Rapid digestion (usually complete within 5 to 10 days) from the top down, without noticeable coagulation and with formation of putrid odor and white or yellowish-white sediment; digested portion usually wine colored at first, later becoming light or dark amber, purple or green, and sometimes viscous.
- b. Same as (a) except that digested portion is amber colored, non-viscous, and the odor suggestive of indol.
- c. Alkaline reaction develops slowly, with no apparent proteolysis and no odor; bluish-gray color and white sediment after 14 to 21 days.
- d. Alkaline reaction develops slowly, with slight digestion and slight putrid odor after 14 to 21 days; color bluish-gray to gray and some reduction after 21 to 28 days.
- e. Slightly alkaline reaction after 7 days, followed by an acid reaction, but usually no coagulation after 21 days; no reduction except at bottom of tube.
- f. Acid ring forms and later acid coagulation from the top down; slight proteolysis and May apple odor sometimes evident.

<sup>2</sup> A classification of the fluorescent organisms in dairy products and related materials is at present being developed.

Although each of the groups probably represents more than one species, the grouping appears to be a logical basis from which to work out a classification of the fluorescent organisms common in dairy products.

The action of the cultures on fat was tested with corn oil and cotton-seed oil, rather than butterfat, because of the convenience of a relatively low melting point fat. Of the 496 cultures, 305 were lipolytic with the nile blue sulfate technic (13). The lipolytic cultures varied considerably in activity. All the cultures which hydrolyzed corn oil also hydrolyzed cotton seed oil; with a few cultures there were slight differences in the extents to which the two fats were hydrolyzed.

The effect of the fluorescent organisms on the flavor of milk and cream held at 5°C. was studied with 27 representative cultures. Of these, 15 digested litmus milk, 7 developed an alkaline reaction without digestion, and 5 produced acid coagulation; 9 of the proteolytic and 1 of the acid-coagulating cultures were lipolytic. The cultures were inoculated into sterilized skim milk and pasteurized (65.6°C. for 30 minutes) whole milk and cream (18% fat); an uninoculated sample of each product was held as a control.

During 18 days at 5°C. each organism produced essentially the same flavor in the three products, except that a slight nutty flavor was evident in some milk and cream cultures but not in the skim milk cultures. With the amount of inoculation used (0.1 ml. of a 24-hour milk culture per 100 ml.), off flavors were not noted until after 10 days in whole milk and cream although they sometimes were evident after 8 days in skim milk. Of the 15 proteolytic cultures, 11 developed a bitter flavor that usually became astringent or quinine-like as holding continued, 2 formed a nutty flavor, while the other 2, probably because of their slow growth, had no effect. One of the acid-coagulating cultures produced a putrid flavor after 12 days, but the other 4 acid-coagulating cultures and the 7 alkali-forming cultures developed only a slight stale flavor in milk and cream after 14 to 16 days. Although 10 of the organisms were lipolytic and 3 of them developed a rancid flavor in butter at 1° to 3°C., none of them produced a rancid flavor in milk or cream.

The protein breakdown in skim milk was studied with four actively proteolytic cultures and with one acid-coagulating culture, using the procedure employed by Long and Hammer (12). At 21°C. the proteolytic cultures greatly increased the soluble nitrogen in the milk; proteolysis was extensive after 2 days and increased regularly throughout the 10-day holding period. The degree of proteolysis varied somewhat with the different cultures. The acid-coagulating culture did not increase the soluble nitrogen during the 10 days. At 5°C. the soluble nitrogen in the milk was not increased significantly after 5 days by any of the cultures, but it was markedly increased after 14 and 21 days with three of the proteolytic cultures.

At 21°C. the distribution of nitrogen in the various fractions was essentially the same with the four proteolytic cultures. Along with the increase in total nitrogen there were increases in amino nitrogen and

in the nitrogen soluble and insoluble in the various reagents except that insoluble in trichloroacetic acid. The amounts of nitrogen in the fractions increased as the incubation period increased, but there was little change in the relationships of the various fractions although the amino nitrogen was fairly constant during the first 5 days and showed a pronounced increase after 10 days. At 5°C. the distribution of nitrogen was much the same as at 21°C., but the amounts were much less. Ammonia nitrogen was not increased during 21 days at 5°C. by any of the organisms.

#### ACTION OF FLUORESCENT BACTERIA ON BUTTER

The general action of 52 selected cultures of fluorescent bacteria on butter was studied as follows: 500 ml. of sterilized cream to which 10 ml. of a milk culture of a test organism had been added was churned, washed with sterile water, and worked in sterile equipment; part of the butter was left without salt, while 2 per cent salt was added to the remainder. With each organism, unsalted butter also was made with addition of 10 per cent butter culture to the cream. All the lots of butter were held in sterile petri dishes at 21°C. and changes in flavor noted during 7 days; the unsalted butter made without butter culture also was held at 1° to 3°C. and changes in flavor noted during 28 days. Acidity of the fat (3) in the unsalted samples made without butter culture was determined at once and after 7 days at 21°C. The data are given in Table 1.

In unsalted butter made without butter culture and held 7 days at 21°C., 47 (90.4%) of the 52 cultures produced some flavor defect, the off-flavors usually being evident after 2 days but sometimes not until near the end of the holding; at 1° to 3°C., off flavors were produced by 21 cultures during the 28 days of holding. Addition of butter culture to the cream or salt to the butter prevented development of flavor defects in the butter at 21°C. for 7 days by some organisms but not by others; with butter culture, 36 (69.2%) of the 52 cultures were detrimental to the flavor of the butter, while with salt 25 (48.1%) cultures produced flavor defects. Off flavors did not develop as rapidly, and commonly were not as pronounced, when butter culture or salt was used as without such additions.

In the unsalted butter made without butter culture and held at 21°C., more than one off flavor sometimes was evident in a sample, and the flavors present often changed as the sample was held. Cheesy or slightly putrid flavors were present in some samples when fairly fresh, but upon further holding only a rancid flavor was evident. Samples which developed a pronounced putrid flavor, however, continued to show this defect throughout the holding period, even when rancidity was evident later. Putrid or putrid and bitter flavors were quite conspicuous in some samples and were very offensive. Some cultures that hydrolyzed corn oil and cotton seed oil and increased the acidity of the fat developed only a putrid flavor in the butter; presumably, the flavor of the free fatty acids was submerged by the pronounced putrid flavor. A rancid flavor was quite intense in the 13 samples that developed only this type of

TABLE 1  
ACTION OF SELECTED CULTURES OF FLUORESCENT BACTERIA ON BUTTER

No. Culture	FLAVOR OF BUTTER AFTER HOLDING 7 DAYS AT 21°C.				ACIDITY* OF FAT OF BUTTER MADE WITHOUT BUTTER CULTURE OR SALT	
	No Butter Culture or Salt	10% Butter Culture in Cream	2% Salt in Butter	28 Days at 1° to 3°C.; No Butter Culture or Salt		
1	putrid	sl. † putrid	good	good	0.70	2.20
2	putrid, rancid	sl. putrid	good	sl. putrid	0.70	3.40
3	sl. putrid	unclean	good	good	0.70	0.75
4	cheesy	sl. rancid	good	good	0.70	1.30
5	putrid, rancid	sl. putrid	good	good	0.70	3.10
6	cheesy	unclean	good	good	0.70	1.60
7	putrid, rancid	sl. putrid	good	good	0.70	2.70
8	rancid	sl. rancid	sl. rancid	sl. rancid	0.80	7.60
9	sl. putrid	good	good	good	0.80	1.00
10	putrid	putrid	sl. putrid	sl. putrid	0.80	2.30
11	rancid	rancid	rancid	sl. rancid	0.80	4.40
12	putrid	putrid	putrid	sl. putrid	0.80	2.50
13	putrid	good	good	good	0.80	0.80
14	rancid	sl. rancid	sl. rancid	sl. rancid	0.33	4.60
15	unclean	good	good	good	0.33	0.35
16	fruity, rancid	good	sl. putrid sl. rancid	sl. putrid sl. rancid	0.33	1.65
17	putrid, fruity	unclean	unclean	unclean	0.33	0.35
18	unclean, fruity	unclean	unclean	good	0.33	0.60
19	rancid	sl. rancid	sl. rancid	rancid	0.33	10.35
20	rancid	rancid	rancid	rancid	0.33	16.90
21	rancid	sl. rancid	sl. rancid	rancid	0.33	8.40
22	fruity	fruity	fruity	good	0.80	0.90
23	putrid	good	good	sl. putrid	0.80	2.70
24	rancid	sl. rancid	sl. rancid	rancid	0.80	10.60
25	rancid	good	good	good	0.80	3.00
26	rancid	sl. rancid	sl. rancid	rancid	0.80	12.00
27	rancid	rancid	good	good	0.33	4.00
28	cheesy, rancid	good	good	good	0.33	2.30
29	fruity, rancid	fruity, rancid	good	good	0.33	1.05
30	good	good	good	good	0.33	0.54
31	fruity, putrid, rancid	putrid, rancid	sl. putrid, sl. rancid	sl. rancid	0.33	1.95
32	good	good	good	good	0.33	0.30
33	putrid, fruity	sl. putrid	sl. putrid	fruity	0.33	0.32
34	putrid, rancid	putrid, rancid	rancid	good	0.43	3.75
35	putrid, bitter	good	sl. rancid	good	0.43	0.95
36	rancid	rancid	rancid	rancid	0.43	11.95
37	good	good	good	good	0.43	0.45
38	good	good	good	good	0.43	0.45
39	good	good	good	good	0.43	0.55
40	fruity	fruity	good	good	0.43	0.45
41	putrid	good	good	good	0.43	0.65
42	putrid, rancid	sl. bitter	good	sl. putrid, sl. rancid	0.43	1.70
43	putrid, bitter	sl. putrid	sl. rancid	sl. rancid	0.43	2.70
44	putrid, bitter	sl. putrid	sl. putrid	good	0.43	0.45
45	putrid, bitter	putrid	putrid	putrid	0.43	0.45
46	putrid, bitter	putrid	putrid	good	0.40	0.50
47	putrid, rancid	sl. rancid	good	putrid, rancid	0.40	2.20
48	rancid	sl. rancid	sl. rancid	good	0.40	5.20
49	rancid	sl. rancid	sl. rancid	good	0.40	2.70
50	putrid, bitter	sl. putrid	good	good	0.40	1.40
51	al. cheesy	good	good	good	0.40	0.80
52	unclean	good	good	good	0.40	0.40

\* — MI. 0.1 N KOH required to neutralize 10 gm. fat.

† sl. — slightly.

defect and was correlated with a high acidity of the fat. A fruity flavor occurred in several samples along with other flavors, but 2 samples developed only this flavor. At the end of the holding the flavors in the samples were putrid, cheesy, or unclean, 13; putrid and bitter, 6; putrid or cheesy and rancid, 7; rancid, 13; fruity and rancid, putrid or unclean, 6; and fruity, 2.

The acidity of the fat was below 1.0 in 20 samples (none of which were rancid), between 1.0 and 2.5 in 13 samples (6 of which were rancid), between 2.6 and 5.0 in 11 samples (9 of which were rancid), between 5.1 and 10.0 in 3 samples (all of which were rancid), and above 10.0 in 5 samples (all of which were rancid).

One culture produced a bluish-purple color on the surface of unsalted butter within 5 days at 21°C. The color darkened somewhat during the holding, becoming bluish-black after 2 to 3 weeks. The coloration did not extend more than 2 to 3 mm. into the butter, but the entire surface of the butter in the container was affected. Another culture developed a salmon-pink color in unsalted butter within 1 week at 21°C.; it extended throughout the entire butter mass and did not change as the sample was held.

#### KEEPING QUALITY TESTS ON FRESH, SWEET CREAM BUTTER YIELDING FLUORESCENT BACTERIA

The 113 samples of fresh, sweet cream butter which were examined for fluorescent bacteria (p. 369) were held at 21°C. and the flavor noted at 2-day intervals for 6 to 8 days. In the original examination of the samples, 39 (34.5%) yielded fluorescent organisms, 22 directly and 17 only after enrichment. At the end of the holding the samples which originally had not yielded fluorescent bacteria were again examined for them; the results were largely negative, and the samples were not considered further.

Of the 39 samples which yielded fluorescent organisms, 19 (48.7%) developed defects at 21°C.; of the 74 samples not yielding such organisms, 26 (35.1%) developed defects. In each group of samples both slight and conspicuous defects were encountered. Among the conspicuous defects in each group cheesiness and rancidity were common; often cheesiness was first evident and then rancidity appeared. Of the samples yielding fluorescent organisms, only one showed a pronounced cheesiness unaccompanied by rancidity; in one sample most of the 27,500,000 non-butter culture organisms per ml. originally present were fluorescent and this butter rapidly became cheesy and rancid at 21°C.

#### DISCUSSION

The wide distribution of fluorescent organisms in dairy products is probably explained by their frequent presence in water, feeds, soil, and barn surroundings, from which they gain entrance to the products or to utensils and equipment coming in contact with them.

The conspicuous action of many of the organisms on dairy products

makes them potential causes of objectionable conditions in these materials; such action involves both the protein and fat. Because of the low growth temperatures of most of the organisms, they can develop at temperatures which might be expected to prevent bacterial action for rather extended periods. It appears that butter is the most susceptible of the common dairy products to serious spoilage by the fluorescent bacteria. Often it is held for rather extended periods at temperatures above the freezing point. The fluorescent bacteria can produce various types of defects in butter, but in agreement with their action on protein and fat, cheesiness and rancidity are especially common. However, both butter culture and salt tend to delay or prevent growth of the organisms.

The frequency with which rancidity, either alone or with some other off flavor, developed in butter made from cream inoculated with fluorescent organisms emphasizes the lipolytic action of these types. With two off flavors present, rancidity frequently is the more prominent because it is so objectionable.

The results of the keeping quality tests on the fresh, sweet cream butter indicate that defects can develop whether or not fluorescent organisms are in the butter; they also show that either with or without fluorescent organisms cheesiness and rancidity are especially prominent defects.

The failure to identify many of the cultures on the basis of published descriptions shows that an adequate classification of the fluorescent, gram-negative, non-spore-forming, rod-shaped bacteria so widely distributed in dairy products is greatly needed. It appears that the changes in litmus milk are a logical basis from which to develop such a classification.

#### SUMMARY

By means of enrichment procedures fluorescent bacteria were found widely distributed in the milk, cream, ice cream, and butter, and also in water and materials from dairy and barn surroundings. Such procedures were much more satisfactory for the detection of fluorescent organisms than direct plating.

The 496 cultures of fluorescent bacteria studied in some detail were gram-negative, non-spore-forming rods, with polar flagellation, which grew well on the common media and were psychrophilic. They produced six general types of changes in litmus milk; these may serve as a basis from which to develop a classification of the fluorescent organisms common in dairy products. Some of the cultures were actively proteolytic and some were not. Many of them hydrolyzed fat.

Selected cultures produced various types of off flavors in milk and cream held 18 days at 5°C., but rancidity was not detected although the selected cultures included some that were lipolytic.

Of 52 selected cultures, 47 (90.4%) produced flavor defects in unsalted butter held 7 days at 21°C. Addition of butter culture to the cream or salt to the butter prevented development of flavor defects by some organisms but not by others; with butter culture 36 (69.2%) of the 52 cultures produced off flavors in the butter, while with salt 25 (48.1%)

cultures produced off flavors. More than one off flavor sometimes was present in a sample, and the flavor often changed as the butter was held. Putrid and rancid flavors were especially common among the off flavors developed.

Of 113 samples of fresh, sweet cream butter (mostly unsalted), 39 (34.5%) yielded fluorescent bacteria, and 19 (48.7%) of these developed flavor defects in keeping quality tests; of the 74 samples not yielding fluorescent organisms, 26 (35.1%) developed defects. Among the conspicuous flavor defects in each group, cheesiness and rancidity were common; often cheesiness was first evident and then rancidity developed.

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# A MICROSCOPIC METHOD OF STUDYING SOIL STRUCTURE

C. LOYAL W. SWANSON<sup>1</sup>

*From the Soils Subsection, Iowa Agricultural Experiment Station<sup>2</sup>*

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## INTRODUCTION

In soil studies employing the microscopic examination of thin sections of soil prepared (3, 5, 6) from representative soil samples, it is desirable to record the microscopic images for future reference and for comparison purposes with other soil samples of various soil types. Such recordings can be made by taking photomicrographs (2, 4, 6, 8) of the thin sections of soil by the usual photographic means (1). However, photomicrography requires the purchase of expensive equipment and the employment of considerable skill and knowledge of photography in the taking of good photographs (1).

In an attempt to record microscopic images of soil quickly and easily, a microprojection apparatus was devised which made possible the tracing of projected microscopic images of soil in the natural structural state as they appear in thin sections. The speed and simplicity of tracing the outlines of pore spaces directly from a projected microscopic image make such a method suitable for wide application.

This qualitative procedure contributes to the quantitative comparisons of pore spaces on the basis of size distribution and volume. Tracings of thin sections make available to others representative images of certain characteristics which are impossible to describe by words alone. However, it is realized that such tracings are not as instructive as actual examination of the object at first hand. The selection of a truly representative specimen of soil in thin sections requires a true concept of the nature of the population represented by the sample. Even without a microscope it is possible to get a macroscopic idea of the quality of the structure of a soil by looking at a well-made thin section. For the best observation, some kind of illumination should be used, such as laying the thin section on the face of the glass of a substage microscope lamp.

## DESCRIPTION AND USE OF THE MICROPROJECTION APPARATUS

The microprojection apparatus is shown in Figure 1. A Spencer petrographic microscope was used in producing the images. Since each Nicol prism wastes about 50 per cent of the light entering it (1), a Zeiss microprojector equipped with a carbon arc was employed for the source of light and for projecting the images onto a ground-glass plate. To facilitate the operation of the hand-fed carbon-arc lamp, the coaxial knobs used

<sup>1</sup> Formerly Teaching Fellow in Soils, now Instructor in Agronomy, Department of Agronomy, University of New Hampshire, Durham, New Hampshire.

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in adjusting the carbons were extended, making them easily accessible during the operation of the apparatus. A table was remodeled by replacing the top with a 20-inch square, ground-glass plate and attaching adjustable legs so that it could be raised or lowered for the purpose of varying the magnification of the projected image. Black cotton flannel cloth held in a conical form by spring steel wire served as a bellows. Exclusion of all stray light permitted the projection of microscopic images in a lighted room although the best images were produced in a dark room. The magnification of the projected image was checked with a stage micrometer graduated to 0.01 mm.

To insure that none of the distortion due to spherical aberration would be included in the microscopic fields traced, a brass washer was inserted in the section of the eyepiece adapted to hold the eyepiece micrometer. The solid part of the washer reduced the diameter of the microscopic field in the eyepiece sufficiently to eliminate the distorted edges. Because it was necessary to rotate the stage in order to differentiate between minerals and pore spaces (6), a bumper device was designed so that the rotating stage could be easily and accurately returned to its original position at the completion of a determination.

Well-prepared thin sections (5) of a thickness of  $0.07 \pm 0.01$  mm. were used in which the outlines of the pore spaces stood out more distinctly than in thin sections of the 0.03 mm. thickness commonly employed for mineralogical studies. The mechanical stage was useful in locating the structure to be traced and in recording for reference purposes the location of the microscopic field for each thin section traced.

In order to obtain a distinct outline on tracing paper of the magnified image appearing on the ground-glass plate, a hard lead pencil was used in tracing the pore spaces. For ease of distinguishing pore spaces from soil minerals and the ground mass, a gypsum plate was inserted in the accessory aperture of the microscope. This caused the pore spaces to appear as a pink color under crossed Nicols and on rotation of the microscope stage, while the crystalline and non-crystalline materials exhibited their characteristic optical properties. From 10 to 20 minutes were required to trace the outlines of a projected microscopic image, depending on the number of pore spaces present and on the number of minerals which had to be distinguished from the pore spaces.

#### RESULTS AND DISCUSSION

Figures 3 to 8 inclusive show the relative sizes, shapes, and distributions of pore spaces for the profiles of the Marshall and Shelby silt loam soils from the Soil Conservation Experiment Stations at Clarinda, Iowa, and Bethany, Missouri, respectively (6). For comparative purposes these characteristics are also presented in Figure 2 in a graphical form from previously determined data (6). It is readily seen that the Marshall (Fig. 2) had a more porous structure throughout the profile than the Shelby. It is apparent that in the surface horizon (Figs. 3 and 4), the Marshall had the larger-sized pores and a greater total number of pores. At

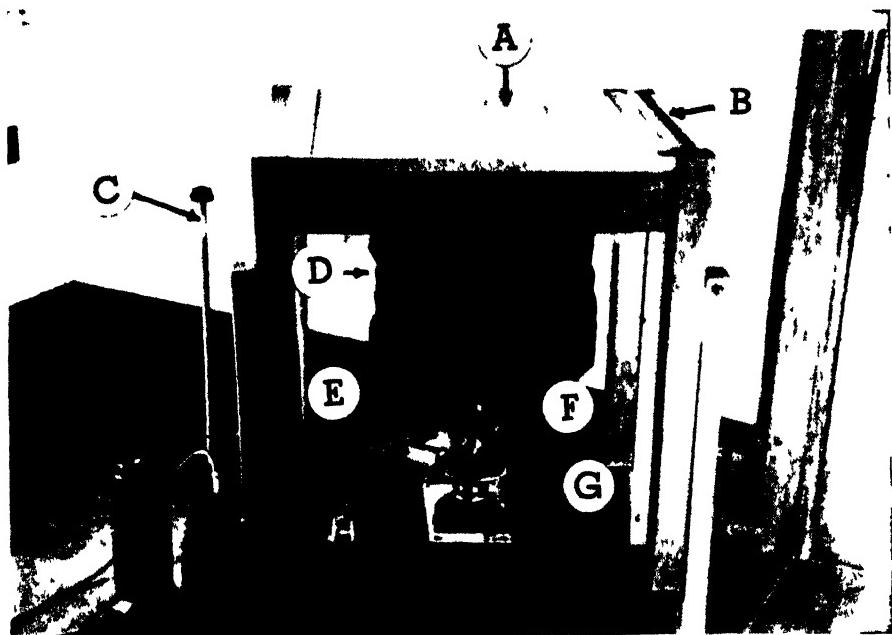


FIG. 1. Microprojection apparatus for tracing projected images of a microscope field. (A) ground-glass plate, (B) adjustable table, (C) coaxial knob extension, (D) bellows, (E) bumper apparatus, (F) petrographic microscope, (G) microprojector.



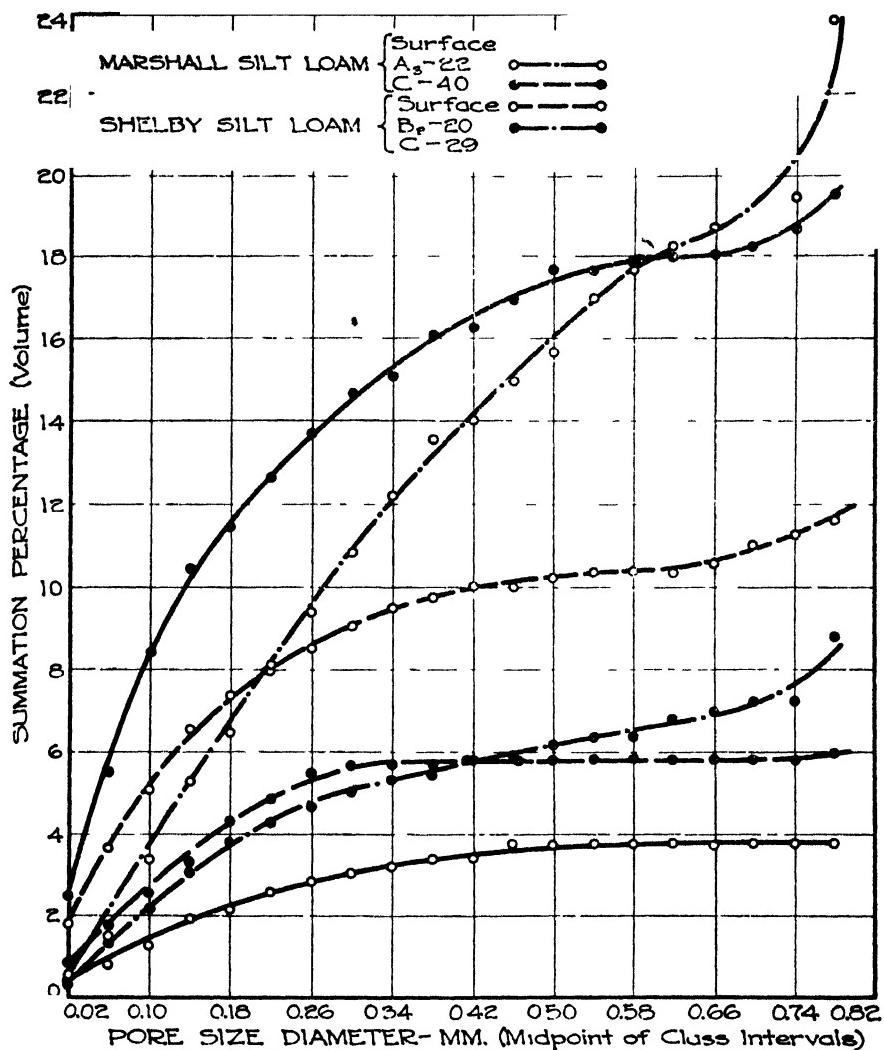


FIG. 2. Size distribution of pore spaces obtained by the micrometric method.

an approximate depth of 20 inches (Figs. 5 and 6), a striking difference is noted in the pore space relationships. The relatively numerous variable-shaped pores in the Marshall at this depth in comparison with the crack-like pore structure in the dense mass of the Shelby suggest a continuation with depth of a larger number of non-capillary pores in the Marshall than in the Shelby. In the C horizons (Figs. 7 and 8) but at different depths, it is evident that the amount of non-capillary pores in the Marshall still continued to be greater than in the Shelby.

A qualitative observation of the accuracy of micrometric analysis (7) is provided by a comparison of the data in Table 1 with Figures 9 and 10.

FIG. 3. Microprojection tracing. Surface horizon, Marshall silt loam. Stippled areas represent pore space. 44.5 X.

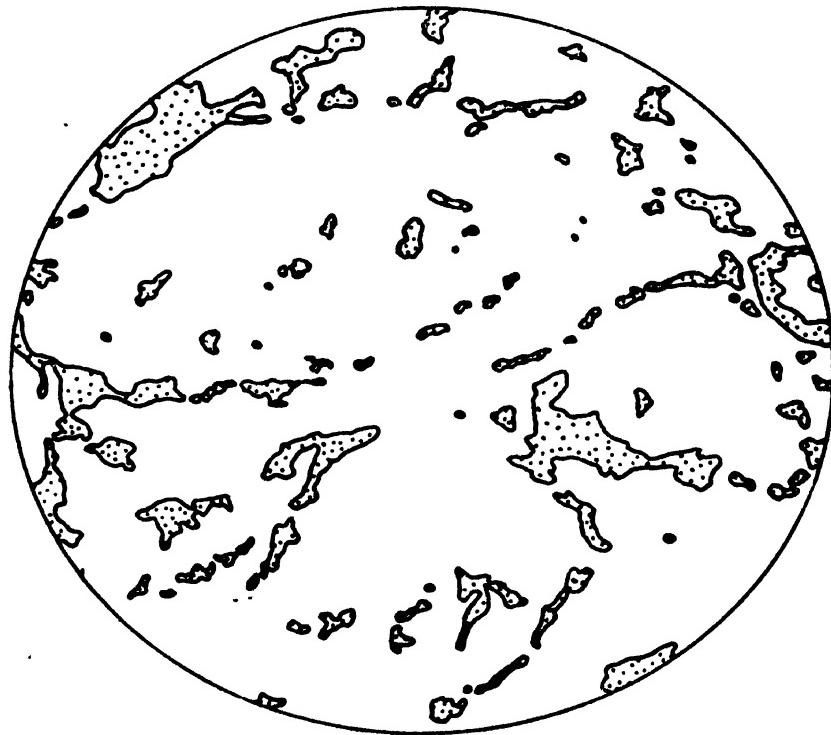
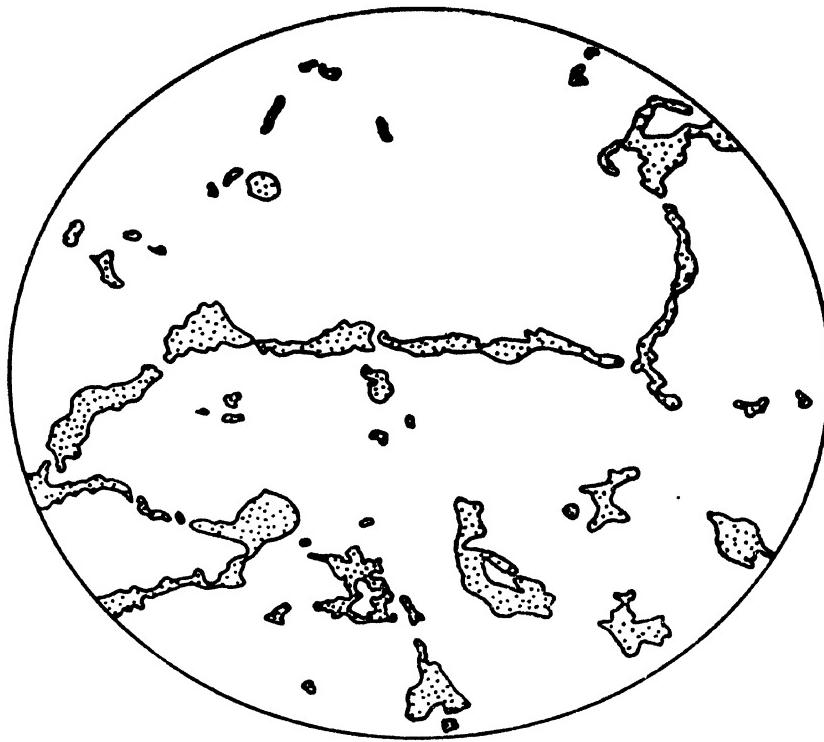


FIG. 4. Microprojection tracing. Surface horizon, Shelby silt loam. Stippled areas represent pore space. 44.5 X.



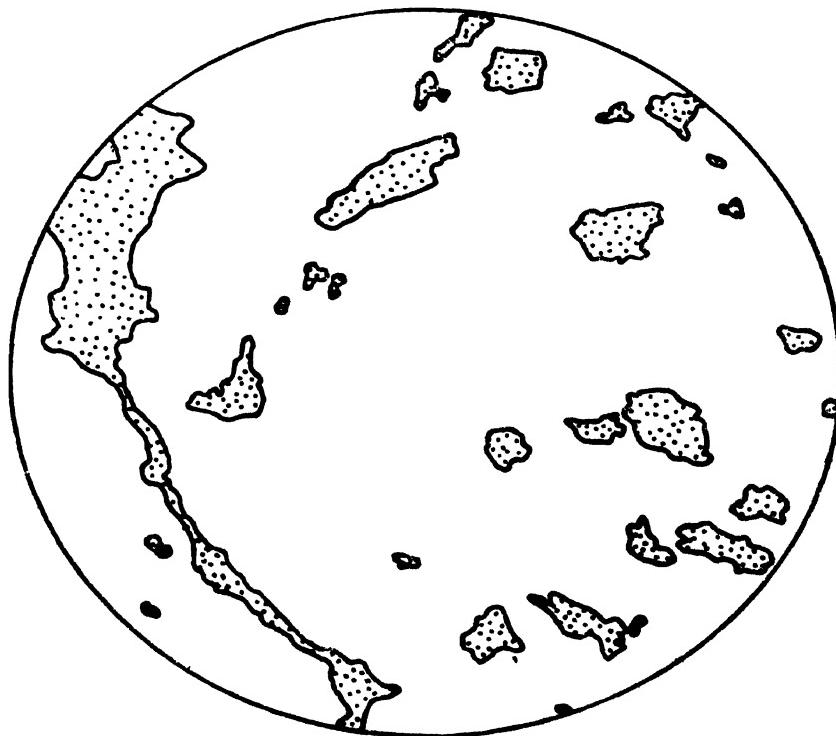


FIG. 5. Microprojection tracing. A-22 inch horizon, Marshall silt loam. Stippled areas represent pore space. 44.5 X.

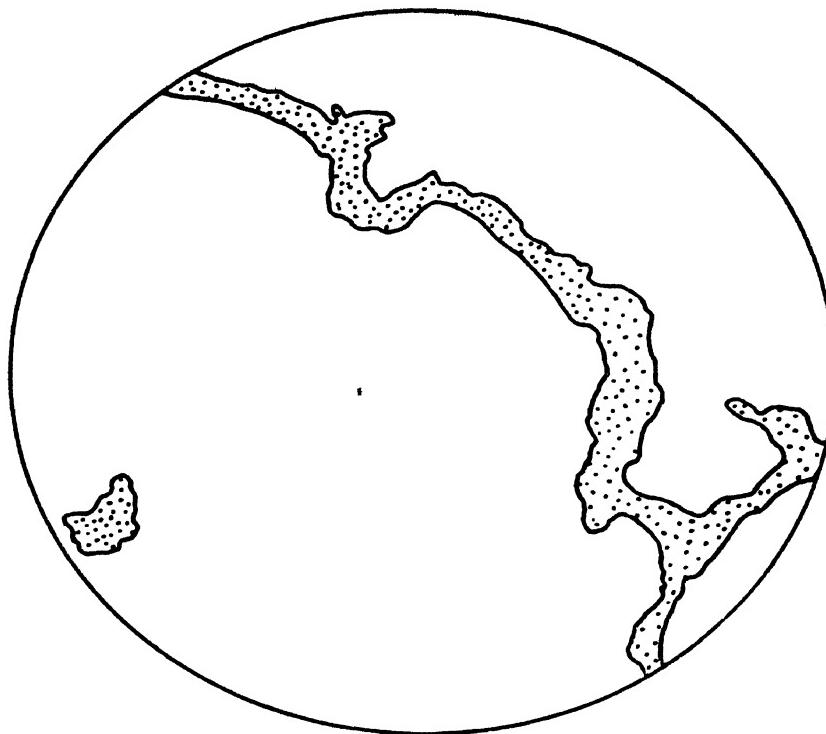


FIG. 6. Microprojection tracing. B-20 inch horizon. Shelby silt loam. Stippled areas represent pore space. 44.5 X.

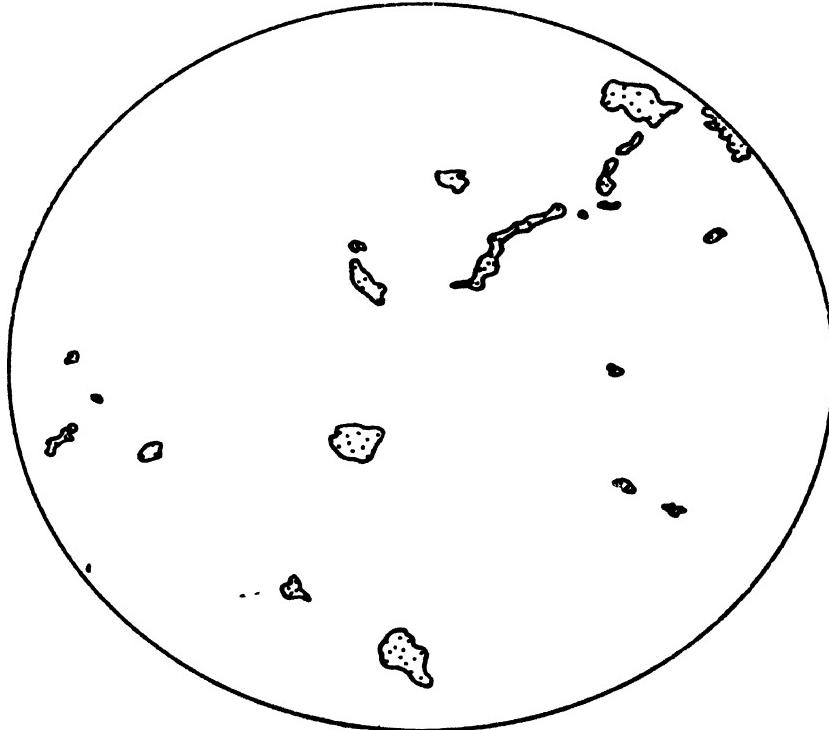


Fig. 7. Microprojection tracing; C-40 inch horizon, Marshall silt loam. Stippled areas represent pore space. 44.5 X.

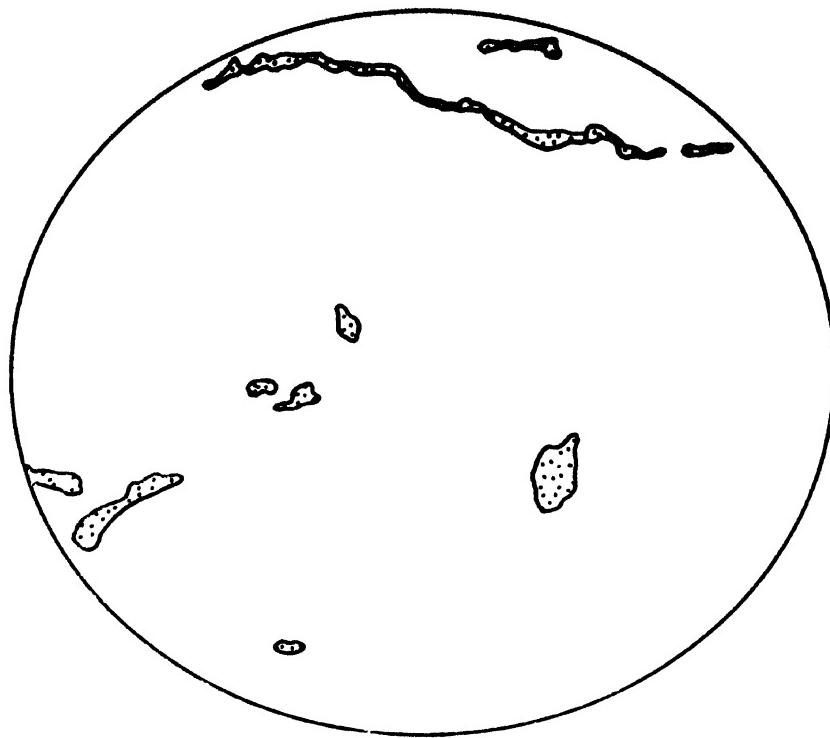


Fig. 8. Microprojection tracing. C-29 inch horizon, Shelby silt loam. Stippled areas represent pore space. 44.5 X.

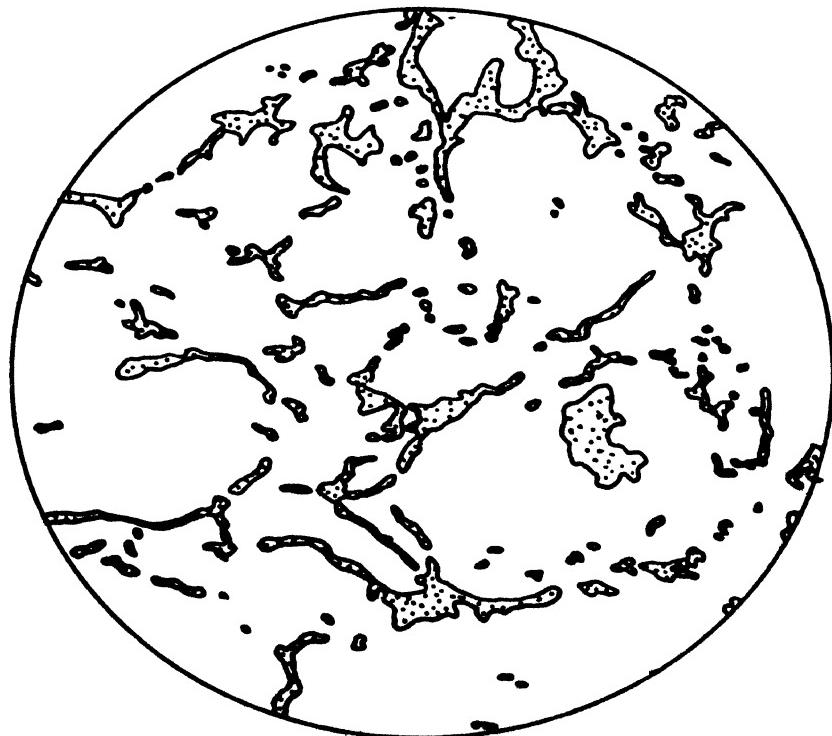


FIG. 9. Microprojection tracing. Surface horizon (virgin), Marshall silt loam. Stippled areas represent pore space.  $44.5 \times$ .

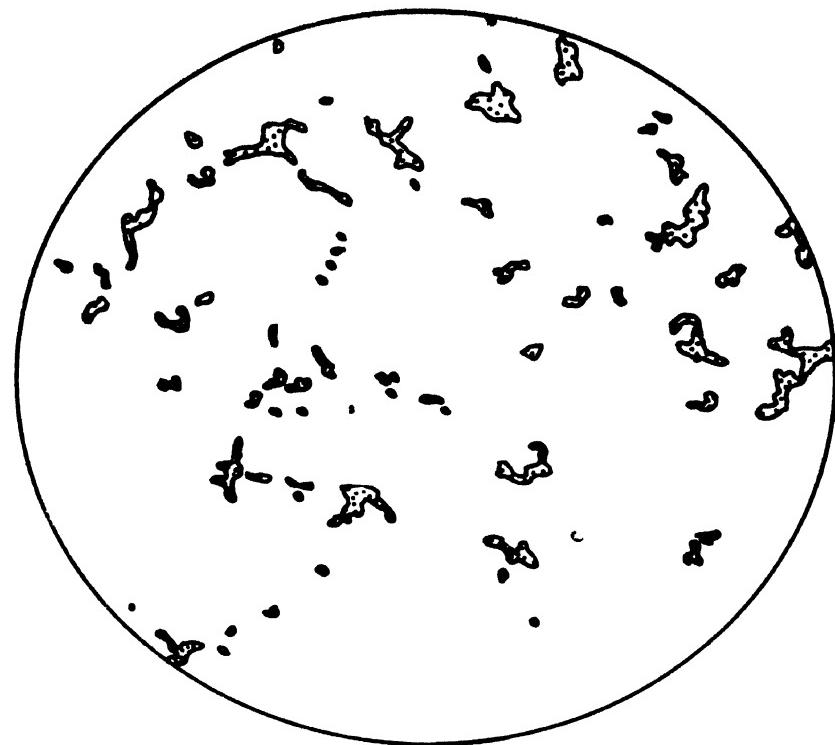


FIG. 10. Microprojection tracing. Surface horizon (cultivated), Marshall silt loam. Stippled areas represent pore space.  $44.5 \times$ .

TABLE 1  
SIZE DISTRIBUTION OF PORES OBTAINED BY THE MICROMETRIC METHOD

MID POINT OF CLASS INTERVAL	MARSHALL SILT LOAM*	
	MICROMETRIC METHOD	
	Virgin†	Cultivated†
mm.‡	%**	%**
0.096	1.36	0.81
0.146	1.38	0.78
0.205	1.36	0.60
0.263	1.01	0.54
0.322	0.73	0.41
0.380	0.62	0.31
0.439	0.51	0.46
0.497	0.23	0.05
0.556	0.30	0.09
0.614	0.38	0.14
0.673	0.27	0.00
0.731	0.29	0.05
0.790	0.21	0.06
0.848	0.23	0.06
0.907	0.25	0.06
0.965	0.20	0.00
1.024	0.07	0.00
1.082	0.15	0.00
1.141	1.41	0.08
Total.....	10.96	4.50

\*Soil samples obtained near Cherokee, Iowa.

†Mean values obtained from four thin sections.

‡Pore diameters.

\*\*Per cent of the total volume of the sample.

These figures are microprojection tracings of the structures exhibited by the virgin and cultivated samples of the Marshall silt loam soil obtained near Cherokee, Iowa. The wide differences in the total amounts of pore space, in the size distribution of the larger pores, and in the outlines of aggregates as suggested by the arrangements of the pore spaces for these two soils are apparent in these two tracings.

In none of the microprojection tracings for the soils studied did there appear to be any directional orientation of the pore spaces.

#### SUMMARY

1. An apparatus was devised which made possible the quick and easy tracing of projected microscopic images of soil in the natural state as they appeared in thin sections. It also facilitated the comparison of the larger-sized pore spaces (non-capillary range) on the basis of shape, size distribution, and volume.

2. Studies of the natural structure of the Marshall and Shelby silt loam profiles as revealed by tracings of projected microscopic images of thin sections of soil and by the micrometric analysis of pore space supplied information concerning their pore space relationships. The porosity

of the Marshall was much greater throughout the whole profile than that of the Shelby. Furthermore, the occurrence of large pores continued to a greater depth in the Marshall, there being many more large pores in the 22-inch sample (A<sub>8</sub>) of the Marshall than in the 20-inch sample (B) of the Shelby.

3. The pore space relationships exhibited by microprojection tracings of a cultivated and virgin surface soil horizon were studied and compared with data obtained by the micrometric analysis of pore space. The results showed that the virgin soil had twice the volume of pores and a higher percentage of large pores than did the cultivated soil.

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# A COMPARISON OF THE ALUMINUM-CHLORIDE AND THE SULFURIC-ACID METHODS FOR QUANTITATIVE ESTIMATION OF WOOL<sup>1</sup>

LOIS W. DALE AND RACHEL EDGAR

*From the Sections of Home Economics (Textiles and Clothing) and Chemistry,  
Iowa Agricultural Experiment Station*

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For at least sixty-five years sulfuric acid of various concentrations has been recommended for quantitative estimation of the wool of cellulosic admixtures, and change in weight of wool by sulfuric acid has been reported by many investigators.

Bayer (3) immersed a half-gram sample of wool 12 hours in 400 volumes of a mixture, 4 volumes of concentrated sulfuric acid and 1 of water, then drained and treated the residual wool 5 hours with a second portion of the acid, and diluted the combined suspensions with 6 volumes of 1:1 ethanol before filtering, washing, and drying the residue at 100°C. This procedure resulted in a 2 per cent loss of wool.

Heermann (11) reported that 5 grams of wool which had been extracted with diethyl ether and with 96 per cent ethanol increased 2 per cent in weight when steeped 3 hours, and 1.5 per cent when steeped 6 hours, in 100 volumes of 80 per cent sulfuric acid in stoppered flasks before dilution, filtration, washing with dilute ammonium hydroxide, and drying to constant weight at 105° to 110°C.; Heermann further reported that the wool lost 5.5 per cent in weight when left in the acid for 24 hours.

Matthews (19) and Green (10) found that wool lost 2.5 per cent of its weight when treated for 12 hours in 1:1 sulfuric acid before dilution with 3 volumes of ethanol and water; 3 determinations by their method in this laboratory (34) resulted in a mean loss of  $33.6 \pm 4.3$  per cent of wool.

Krais and Biltz (15) heated wool to 50°C. in 44 per cent sulfuric acid and allowed it to remain in the cooling bath for 24 hours; 4 determinations by their method in this laboratory (7) resulted in a mean loss of 31.7 per cent of wool. Lloyd and Priestley (18) used acid of this concentration for 20 minutes at 50°C. to remove cotton, and for 24 hours at 25°C. to remove regenerated cellulose from mixtures containing wool; in this laboratory (7) 6 determinations by the former method resulted in a loss of 1.5 per cent of wool and 6 by the latter method, but using 62 per cent sulfuric acid, yielded a loss of 35.8 per cent. A mean increase of but 0.04 per cent (from - 0.5 to + 0.1) for wool treated 3 hours with 80 per cent sulfuric acid according to Schulze's method has been reported by several analysts (29, 13, 14, 16, 17, 8, 12) although VierTEL (31) obtained an 8

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TABLE I  
A COMPARISON OF THE ALUMINUM-CHLORIDE AND THE SULFURIC-ACID METHODS FOR QUANTITATIVE ESTIMATION OF WOOL

TREATMENT OF WOOL	PARALLEL DETERMINATIONS	RESIDUAL WOOL				Sulfate Sulfur Percentage of Wool
		Weight	Ash	Percentage of Wool	Total Sulfur Percentage of Wool	
A. None.....	6	100.0	.....	0.10 (0.01)*	3.76 (0.01)	0.30 (0.01)
B. Aluminum-chloride method.....	2	98.7 (0.4)	.....	.....	3.46 (0.03)	none
	6	98.8 (0.2)	0.43 (0.15)	.....	.....	.....
	6	98.8 (0.4)	.....	.....	.....	.....
	6	100.1 (0.4)	.....	.....	.....	none
	6	100.7 (0.6)	.....	.....	.....	.....
	6	101.0 (0.3)	1.04 (0.11)	.....	.....	.....
C. Sulfuric-acid method.....	2	99.8 (0.4)	0.05 (0.01)	.....	4.82 (0.07)	.....
	6	100.4 (0.2)	.....	.....	.....	.....
	6	101.2 (0.2)	.....	.....	.....	.....
	6	101.4 (0.6)	.....	.....	5.04 (0.15)	1.50 (0.07)
	6	.....	.....	.....	.....	1.51 (0.09)

\*Mean deviations are within parentheses.

per cent and Schaeffer (28) a 10 per cent increase in weight for wool with this method.

Ryberg (27) reported gains of from 1.8 to 6.8 per cent for wool treated at 30°C. for 15 minutes with 70 to 85 per cent sulfuric acid. The use of 70 per cent sulfuric acid has been further modified (2b, 2c, 1a, 1b, 2d).

Yamada and Ikoma (33) and Schaeffer (28) favor the aluminum-chloride method (2a, 22, 23, 20, 24, 25, 26, 2c) described by Viertel (31) as giving results of close concordance and by Skinkle (30) as not so satisfactory.

Since our work was done, Weidenhammer, Prisley, and Ryberg (32) have reported an inter-laboratory comparison of the sulfuric-acid and the aluminum-chloride methods based on weight of residual wool. As reported in their study the change in weight of an all-wool fabric by the aluminum-chloride method varied from -2.3 to +1.4 per cent among 25 determinations and for the 5 sets of 5 determinations the means ranged from -2.0 to 1.1 per cent; change in weight of this all-wool fabric by the sulfuric-acid method varied from -3.6 to +2.8 per cent among 25 determinations, and for the 5 sets of 5 determinations the means ranged from -2.9 to +2.4 per cent. Weidenhammer, Prisley, and Ryberg concluded that both methods were sufficiently accurate and precise to be useful but that, because it was more convenient and less time consuming, the sulfuric-acid method was to be preferred.

The aluminum-chloride and the 70 per cent sulfuric-acid methods are compared in this study by their effects on the weight, ash, total sulfur, and sulfate sulfur of the residual wools (Table 1).

## EXPERIMENTAL PROCEDURE

### PREPARATION OF WOOL

Plain-woven unscoured wool which contained neither sulfite sulfur (9) nor selenium (6) was cut for analysis and extracted continuously 20 hours with sulfur-free benzene in a Soxhlet extractor, dried in air at room temperature, washed in 100 volumes of 0.1 per cent saponin for 5 minutes at 40°C., rinsed 5 times in distilled water at room temperature, dried, again extracted continuously with benzene for 20 hours, dried, rinsed 8 times in water, and dried at room temperature (5).

### ALUMINUM-CHLORIDE TREATMENT OF WOOL

A 5-gram sample of this wool was dried until constant at 105° to 110°C. and immersed 10 minutes in 40 volumes of a boiling solution, 9 grams of aluminum-chloride hexahydrate per 100 milliliters of water. The residue was drained, dried at 105° to 110°C. for 2 hours, rubbed thoroughly in an attempt to remove all the powdery precipitate, rinsed in 100 volumes of dilute hydrochloric acid (1 part by volume of concentrated hydrochloric acid diluted with 9 parts of water), washed free of chloride, dried at room temperature (2c), and then until constant at 105° to 110°C. before analysis for ash, total sulfur, and sulfate sulfur. Six parallel

blank determinations by this aluminum-chloride method yielded a. wool,  $0.07 \pm 0.01$  per cent ash and  $99.9 \pm 0.2$  per cent the weight original wool.

#### SULFURIC-ACID TREATMENT OF WOOL

A 5-gram sample of wool was dried until constant at  $105^\circ$  to  $110^\circ\text{C}.$ , immersed 10 minutes in 100 volumes of boiling sulfuric acid (1 per cent hydrogen sulfate by weight), drained, worked 15 minutes at  $38^\circ\text{C}.$  in 100 volumes of sulfuric acid (70 per cent hydrogen sulfate by weight), drained, washed with cold water, immersed in 2 per cent sodium hydrogen carbonate at room temperature for 5 minutes, washed free of sulfate, and dried first at room temperature and then at  $105^\circ$  to  $110^\circ\text{C}.$  until constant in weight before analysis for ash, total sulfur, and sulfate sulfur.

#### ASH

A 5-gram sample of wool was placed in a porcelain crucible and ignited until constant at the red heat of an electric furnace,  $600^\circ$  to  $700^\circ\text{C}.$

#### TOTAL SULFUR

A 5-gram sample of wool was dissolved in 100 milliliters of a solution, 1 part nitric acid and 2 parts water, in a covered casserole on a steam plate. After the addition of 100 milliliters of Benedict-Denis reagent (4) the mixture was evaporated to dryness, heated to dull red for 10 minutes, dissolved in 100 milliliters of 10 per cent hydrochloric acid, and filtered. This filtrate was diluted to 375 milliliters and boiled during the dropwise addition of 25 milliliters of 10 per cent barium chloride. The precipitate was digested 15 hours on a steam bath, filtered into a weighed Gooch crucible, washed free of chloride, dried in an oven at  $105^\circ$  to  $110^\circ\text{C}.$ , and ignited at  $600^\circ$  to  $700^\circ\text{C}.$  in an electric muffle furnace for 25-minute periods until of constant weight. Blank determinations were made with the reagents.

#### SULFATE SULFUR

A 5-gram sample of wool was dissolved in 50 milliliters of 30 per cent hydrochloric acid on a boiling water bath, cooled, diluted with 50 milliliters of water, and filtered. The filtrate was brought to boiling for precipitation of its sulfate as described before.

#### SUMMARY

1. The aluminum-chloride and the 70 per cent sulfuric-acid methods for estimation of the wool of cellulose admixtures have been compared by their effect on the weight, ash, total sulfur, and sulfate sulfur of the residual wool.
2. The change in weight of wool by the aluminum-chloride method varied from  $-2.1$  to  $+1.9$  per cent among 32 determinations; for 5 sets of 6 parallel determinations the means ranged from  $-1.2$  to  $+1.0$  per cent. The sulfate sulfur of the wool was removed by this method and the ash in-

\* From  $0.10 \pm 0.01$  to  $0.43 \pm 0.15$  per cent for one set and to  $1.04 \pm 0.11$  for another set of parallel determinations.

3. The change in weight of wool by the sulfuric-acid method varied from  $-0.6$  to  $+2.4$  per cent among 18 determinations; for 3 sets of 6 parallel determinations the means ranged from  $0.4$  to  $1.4$  per cent. The sulfate sulfur of the wool was increased from its original  $0.30 \pm 0.01$  to  $1.51 \pm 0.08$  per cent, the total sulfur from  $3.76 \pm 0.01$  to  $4.82 \pm 0.07$  for one set, and to  $5.04 \pm 0.15$  for another set of 6 parallel determinations although the ash was lowered from  $0.10 \pm 0.01$  to  $0.05 \pm 0.01$  per cent.

4. With the proportion of wool at a maximum, these data describe the 70 per cent sulfuric-acid method as the better of the 2 methods.

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# NEW ANTS FROM MINNESOTA, IOWA, AND WISCONSIN

WILLIAM F. BUREN

From the Department of Zoology and Entomology, Iowa State College

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Iowa, Minnesota, and northern Wisconsin are regions which seem to have been neglected by American myrmecologists. Thus it is not surprising that new ants, especially parasitic species which tend to be rare, sporadic, or circumscribed in distribution, can be collected in these regions.

Holotypes and most of the paratypes are to remain in the author's collection. A few paratypes of each species will be deposited in the National Museum and the Iowa State College collection. There are also a few paratypes in the collection of Herbert T. Dalmat of New York City, who accompanied the writer on the collecting trip during which most of these species were taken.

## *Formica reflexa*, n. sp.

### WORKER

Length, 4.65-5.27 mm.

Head, excluding the mandibles, a little longer than broad, somewhat broader behind than in front, with convex posterior margin, evenly and broadly rounded posterior corners, and moderately convex sides. Mandibles 7-toothed. Maxillary palpi approximately .64 mm. long. Clypeus feebly carinate, subangularly produced in front. Apex of frontal area indistinct. Frontal carinae short, about 1½ times as long as the diameter of the antennal foramina, evenly diverging behind. Eyes hairy. Funicular joints 2-10 subequal in length, the penultimate never shorter than the second. Pro- and mesonotum moderately convex in profile, the middle of the mesonotum straight. Epinotum rather angulate in profile, the base and declivity nearly straight and subequal in length. Petiole small, anterior surface convex, the posterior surface less so; superior border unusually blunt, approaching *Polyergus* species in this respect; the sides and superior border at least slightly convex when seen from behind. Entire body more slender and less robust than *F. dakotensis*.

Head, legs, and gaster feebly shining, clypeus and thorax subopaque. Shagreening on head, thorax, and gaster fine and dense, concentrically set on the pro- and epinotum.

Erect hairs sparse; rather short on most parts of the body, particularly on the pro- and mesonotum where they are about .03-.05 mm. in length. An occasional hair slightly clavate. No erect hairs on the scapes, femora, or tibiae, few on the front and mesonotum, sparse on the dorsum of the gaster. Pubescence extraordinary, consisting of fine hairs almost as long as some of the erect hairs, often growing out from the surface suberectly but sharply reflexed in the middle so that the distal ends strike the surface. Reflexed pubescence most obvious on the gaster.

Pubescent hairs numerous but not dense, nowhere concealing the surface, the distance between the bases of adjacent hairs about .03 mm.

Head and thorax reddish brown, the vertex, occiput, pro- and mesonotum sometimes feebly infuscated. Gaster black to the naked eye, under magnification often deep brown with black posterior borders to the segments. Antennae nearly black, legs dark brown.

#### FEMALE

Length, 5.41-6.32 mm.

Head, excluding the mandibles, as broad as long (about 1.14 mm.), broader behind than in front, with the posterior border convex except in the middle where it is slightly excised, the posterior corners evenly rounded, and the sides nearly straight. Mandibles 7-toothed. Clypeus ecarinate or nearly so. Frontal area rather indistinct. Frontal carinae diverging behind; short, about 1½ times as long as the diameter of the antennal foramina. Funicular joints 2-10 subequal in length, the penultimate joint as long as or slightly longer than the second, and only slightly shorter than the fourth. Eyes hairy, little or no larger than in the worker. Thorax five-sixths as wide as the head, approximately .93 mm. across the wing insertions. In profile the pronotum and epinotum more sloping than in *F. dakotensis*. Petiole low and even blunter than that of the worker, the sides and superior border moderately convex when seen from behind. Wings about 5.5 mm. long. Whole body much more slender and less robust than *dakotensis*.

Entire body very shining, without shagreening, marked only by the punctures from which the pilosity and pubescence arise.

Erect hairs not very numerous on any part of the body, most abundant on the dorsum of the thorax, sparse on the head and gaster. Hairs on the dorsum of the thorax often slightly clavate. Pubescence unique, consisting of fine hair a little shorter than the erect hairs, growing out from the surface suberectly, but sharply reflexed in the middle so that the distal ends strike the surface. Reflexed pubescent hairs numerous but not dense on all body surfaces, the bases of adjacent hairs about .03 mm. apart. Pubescent hairs flexuous on the legs but usually not sharply reflexed.

In mature specimens, head deep blackish brown, most of the thorax dark brown, the scutellum nearly black, the epinotum lighter. Gaster black to the naked eye. Legs dark brown. Antennae black. In dorsal aspect, the insect has a dark, almost black appearance.

#### MALE

Length, 6.54-7.49 mm.

Head, excluding the mandibles, distinctly broader than long, the posterior border broadly rounded. Mandibles indistinctly 3-toothed. Eyes hairy. Funicular joints 2-10 gradually decreasing in length, the second about one-fourth again as long as the penultimate, and as long as the first. Whole body more slender and less robust than in *F. dakotensis*. Petiole low and blunt, the superior border straight or slightly convex when seen from behind.

Head and thorax subopaque, gaster feebly shining.

Erect hairs short, numerous on the dorsum of the thorax, much sparser on the head and gaster. Pubescent hairs long, straight, but usually subappressed to suberect so that it is difficult to distinguish between pilosity and pubescence. Pubescence moderately sparse, nowhere concealing the surface; denser, however, than on the worker and female.

Color black, legs brown.

Described from 53 virgin females (most of them partially callow), 41 males, and 129 workers taken from a small, obscure, roadside nest near Hibbing, Minn., August 13, 1941. This species was also found at Owatonna, Minn. (23 virgin females and 33 workers, August 17, 1941, and 10 workers, July 6, 1940); at Jenkins, Minn. (24 workers and 9 males, August 11, 1941, and 18 workers, July 10, 1940); and at Spirit Lake, Iowa (20 workers from four small nests, June 12, 1940, and 5 workers, July 15, 1940).

Differing from all other species of *Formica* known to the writer by having the pubescence reflexed and the petiole unusually blunt. Among the species of *Formica*, *reflexa* seems most closely related to *dakotensis* Emery. *F. reflexa* differs from this species mainly by having the sides of the head less convex, reflexed rather than straight pubescence, smaller size, and the petiole very blunt rather than cuneate in profile and convex above rather than truncate or excised when seen from behind.

*F. ferocula* is described by Wheeler as having a very blunt petiole. This may possibly be as blunt as that of *reflexa*. *F. ferocula* differs from *reflexa* by having the head as broad as long, the posterior border of the head feebly excised instead of convex, the first four funicular joints longer than the penultimate, the epinotum with a slightly convex base and distinctly concave declivity rather than with a straight base and declivity, the erect hairs numerous on parts of the thorax and absent on the posterior portions of the head, and by not having the pubescence reflexed. *F. ferocula* was thought by Wheeler to be allied to the *ciliata* group of species. Thus it probably is not closely related to *reflexa*.

Although the females of *reflexa* are very small and definitely microgynous, this species does not seem closely related to any of the species of the *microgyna* group. *F. reflexa* can be distinguished from all members of this group by the unusual shape of the petiole, the reflexed pubescence, the proportions of the funicular joints, and the very small, rather uniformly sized worker cast.

The very small, uniform size of the worker, unlike most species of the *rufa* group, and the small, blunt petiole (as if atrophied) seem to show a highly developed parasitism.

Of the seven nests that the writer has found, none were without numerous workers of the host species, *F. fusca subsericea*, the *subsericea* workers in all cases outnumbering the *reflexa* workers at least several to one. This immediately suggests permanent social parasitism of the dulotic type, like that of *Polyergus* species. That *reflexa*'s relationship with *subsericea* is not of the dulotic type is evidenced by the fact that *reflexa* lacks the clypeal notch characteristic of all species of *Formica* which engage in dulosis, and that the workers are much smaller, weaker,

and even more timid than their hosts. Upon opening their nests, the *reflexa* workers disappeared as quickly as possible, leaving the *subsericea* workers to defend the nests.

*F. reflexa* can hardly be a temporary social parasite. In this type of parasitism it is usually very difficult to find mixed colonies.

If *reflexa* is a permanent social parasite of the nondulotic type, the females should exhibit very strong inquilinous characteristics, for it would be fatal to seek adoption in an incipient or depauperate colony as do all other parasitic *Formica* species. Instead, adoption must be secured in a flourishing colony which can furnish enough host workers to rear *reflexa* broods for at least two or three seasons. However, if the host queen is retained as in *Strongylognathus testaceus* of Europe, incipient nests could be chosen.

Much experimentation should be done with this species to determine its exact relationship with *F. subsericea*.

### *Formica fossaceps*, n. sp.

#### WORKER, MAXIMA

Length, 6.65–7.71 mm.

Head, excluding the mandibles, slightly longer than broad, narrower in front than behind, with feebly excised or straight posterior border and nearly straight sides. Clypeus evenly rounded in front, ecarinate or distinctly carinate only at the extreme front. Clypeal fossae strikingly deep; antennal fossae also rather deeply impressed. Maxillary palpi short. Apex of frontal area rounded. Frontal carinae diverging, about twice as long as the diameter of the antennal foramina. Frontal furrow indistinct, ending in a shallow pit level with the eyes. Eyes hairless. Scapes surpassing posterior lateral corners of the head by nearly two-fifths of their length. Funicular joints gradually decreasing in length toward the penultimate, the second approximately four-fifths as long as the first and about one-third again as long as the penultimate, the third slightly shorter than the second; joints 5–8 rather unusual in appearance, each narrow at the base but swollen to more than 1½ times the base width near the apex. To the naked eye these joints are somewhat bead-like.

Pro- and mesonotum not as convex as in *F. rufa obscuriventris*. Meso-epinotal depression not as deep. Epinotum in profile with subequal base and declivity, the base slightly convex, the declivity feebly concave. Petiole large, higher than the epinotum, in profile cuneate, the superior border very sharp. Seen from behind, the petiole broad, measuring up to .77 mm. wide, and strongly subangularly or convexly produced upward.

Head and legs moderately shining, gaster more shining but not as highly polished as that of the female, the shagreening on the gaster very fine and superficial. Thorax subopaque.

Erect hairs absent on most body surfaces, present on the dorsum of gaster near the apex, venter of gaster, venter of petiole, and coxae. Pubescence very short and sparse, especially on the head and gaster,

rather dense only on the base of gaster, petiole, epinotum, and appendages.

Head and thorax ferruginous, appendages brown, entire gaster deep black.

#### WORKER, MEDIA

Length, 5.23-6.62 mm.

Differing from the major in having the head straight or slightly convex behind, the clypeal fossae a little less deep, the basal funicular joints shorter in proportion to the penultimate, the middle joints less bead-like, and the head and thorax often infuscated.

#### WORKER, MINIMA

Length, 3.70-4.58 mm.

Differing from the major in having the head definitely longer than broad and slightly convex behind, the clypeus distinctly carinate, the clypeal fossae more normal in depth, funicular joints 2-10 more nearly subequal in length, and the middle joints not bead-like. Also differing in having the body a little less shining and the head and thorax heavily infuscated.

#### FEMALE

Length, 7.19-7.84 mm.

Although little shorter than the females of *F. rufa obscuriventris*, strikingly more slender and much less massive than this species.

Head, including the mandibles, triangular in shape, the outer borders of the mandibles nearly aligned with the sides of the head; excluding the mandibles, slightly longer than broad (about 1.38 mm. wide), one-third again as wide behind as in front, with straight posterior border and sides. Clypeus evenly rounded in front, ecarinate. Clypeal fossae extraordinarily deep, the middle part of the clypeus between them strongly convex in transverse section. Frontal carinae diverging, approximately twice as long as the diameter of the antennal foramina. Frontal furrow very short and indistinct, not reaching beyond the frontal carinae. Funiculi much as in the major worker, but joints 5-8 not as bead-like and the second joint only a little shorter than the first. Eyes hairless, a little larger than those of the worker. Thorax narrower than the head, about 1.2 mm. wide. Petiole large, cuneate in profile, with sharp superior border, sub-angularly produced upward when seen from behind.

Entire body, especially the gaster, glabrous and extremely smooth. Thorax a little less shining than the head and gaster. Shagreening on the gaster scarcely perceptible, the surface marked only by the very sparse, minute punctures from which the pubescence arises.

Pilosity entirely lacking on all dorsal regions of the body; some slender, scattered hairs on the venter of the gaster and coxae. Pubescence very fine, short, and sparse, about .01-.02 mm. in length, rather dense only on the base of the gaster and appendages; extremely sparse on the head.

Head and thorax light brownish red. Gaster entirely deep black except at the extreme base. Head lightly infuscated behind the eyes.

Mesonotum heavily infuscated in a median frontal spot and along the parapsidal sutures. Scutellum and metanotum black.

**MALE**

Length, 8.06 mm.

Head, excluding the mandibles, distinctly broader than long, much broader behind than in front. Mandibles feebly 3-toothed. Clypeal fossae very deep. Funicular joints 2-10 gradually decreasing in length, the penultimate a little over one-half as long as the second, the second one-fourth again as long as the first. Petiole in profile, with convex anterior and flat posterior surfaces; seen from behind, subrectangular, the superior border sinuate but nearly truncate, the sides almost straight.

Head and thorax mainly opaque, the declivity of the epinotum glabrous; gaster feebly shining.

Erect hairs nearly absent on the dorsal surfaces of the body, a few on the gula, profuse on the venter of the gaster toward the apex. Pubescence sparse, but longer and much denser than in the worker and female.

Color black, the antennae dark brown, legs light brown.

Described from 12 females, 93 workers, and 1 male taken from a nest in wooded pasture near Winterset, Iowa, June 27, 1941. This nest was located around the base of a bush, plant debris being used in the construction of a low dome. Immediately under this dome were the brood, workers, and sexual forms.

This species seems definitely related to *F. rufa* and its numerous subspecies and varieties. It differs from all of them, however, by the paucity of both pilosity and pubescence, the very shining surface, and the depth of the clypeal fossae. *F. rufa obscuriventris* seems to be most closely related in the structure of the clypeal fossae and the funiculi. *F. fossaceps* can be distinguished from *obscuriventris* by the smaller size of the female, the more shining surface of both female and worker, the deeper clypeal fossae, the more bead-like funicular joints, the ecarinate clypeus, and by the lack of pilosity on the dorsal surfaces of both female and worker. In addition *fossaceps* has a somewhat differently shaped head.

From *rufa integra*, an eastern North American form with very sparse pilosity, *fossaceps* can be differentiated by the much deeper clypeal fossae, the proportions of the funiculi, and the shining integument. From *rufa mucescens*, a Colorado form with females of about the same size, *fossaceps* can be distinguished by the shining rather than opaque integument, and very sparse rather than very dense pubescence. The writer has not seen specimens of *mucescens* and thus cannot make any statements regarding the depth of the clypeal fossae. *F. fossaceps* can be separated from *rufa integroides* varieties *haemorrhoidalis* and *ravida*, two Rocky Mountain forms without pilosity, by its much less robust queen, shining integument, very sparse pubescence, and by the unusual clypeal fossae and funicular joints.

*F. fossaceps* is not closely allied to any species of the *microgyna* group, all of which have much smaller females, are more or less pilose,

and have the clypeal fossae more normal in depth. Although *fossaceps* resembles *dakotensis* and its varieties in the smoothness of its integument, it is not closely related to the latter species as shown by the divergent shapes of the head and petiole.

*F. fossaceps* is probably a temporary social parasite of *F. fusca subsericea*.

**Lasius (Acanthomyops) pubescens, n. sp.**

**WORKER**

Length, 3.35–4.06 mm.

Head, excluding the mandibles, as long as broad (about .88 mm.), with straight or feebly convex posterior border and moderately convex sides. Mandibles with 6 strong teeth and 1 or 2 indistinct denticuli, basal borders edentate. Front convex, the frontal furrow absent. Scapes surpassing the posterior lateral corners of the head by one-seventh of their length. Funiculi feebly incrassated, the penultimate joint slightly longer than broad; last joint broader than the penultimate, and a little longer than the ninth and tenth taken together. Eyes hairy, with about 45 facets. Maxillary palpi short, 3-jointed. Pro- and mesonotum moderately convex. Meso-epinotal depression shallow. Epinotum without distinct base or declivity, evenly rounded. Petiole small, narrow, with a transverse, truncate superior border which may be slightly excised but not notched, blunter than in *L. (A.) interjectus* and *L. (A.) claviger*.

Head, thorax, and gaster strongly shining except where somewhat obscured by the pubescence.

Erect hairs short and sparse, shorter and less flexuous than on *interjectus*, lacking, however, only on the scapes, tibiae, and middle and hind femora. Hairs scattered over the surface of the gaster as in *claviger*; more sparse than in *claviger*, however. Pubescence very dense for *Acanthomyops*. Pubescence a little more abundant on the head than on *interjectus*; sparser on the thorax than on the head, but, nevertheless, much more dense than on *interjectus*; dense on the gaster, somewhat concealing the surface.

Color varying from light to brownish yellow, the head never reddish.

**FEMALE**

Length, 4.79–5.18 mm.

Head, excluding the mandibles, a little longer than broad, with feebly excised posterior border and slightly convex sides. Mandibles 6-toothed, basal borders edentulous. Clypeus weakly subangulate in front. Frontal carinae short, scarcely distinct. Eyes hairy, small but quite convex, situated a little farther to the sides than in *interjectus*. Scapes thickened distally, surpassing the posterior lateral corners of the head by one-seventh of their length. Funiculi moderately incrassated, joints 2–9 as broad as or only slightly broader than long, the penultimate joint about as long as broad, the second joint slightly over one-half as broad as the penultimate. Maxillary palpi short, 3-jointed. Thorax distinctly less broad than the head; with about the same shape as *inter-*

*jectus*; the mesothorax a little less flattened dorsally. Petiole small, in profile triangular, with nearly straight anterior and posterior surfaces, the superior border blunt; seen from behind, narrow, the superior border imperceptibly notched and truncate. Femora and tibiae not flattened. Wings about 5.5 mm. long.

Thorax shining, head less so; gaster subopaque, the surface obscured by the dense pubescence.

Pilosity of about the same arrangement as in *interjectus*, but much sparser, much shorter and less flexuous. Hairs very few on gula, front, occiput, and petiole, none on the vertex. A few hairs unevenly distributed along the posterior borders of the gastric segments, a few also scattered over the surface of the gaster. Pubescence particularly dense on the gaster, less dense on the head, sparser on the dorsum of the thorax.

Color dark grayish brown, the appendages lighter.

Described from 4 females and 113 workers taken from a sandy, low mound nest in open woodlands near Jenkins, Minn., August 11, 1941. Since no males and but few females could be found, it is probable that most of the sexual forms had already left the nest.

*L. (A.) pubescens* belongs with the *interjectus* group of species as shown by the long scapes and feebly incrassated funiculi. However, it does not seem closely related to the typical *interjectus*, the queens differing considerably in size, color, pubescence, and pilosity. It may be more closely related to *interjectus mexicanus* which has a queen of equal size. Judging from Wheeler's description of the latter, the queen of *pubescens* can be distinguished definitely from that of *mexicanus* by its different color, shorter wings, and probably also by denser pubescence. The worker of *pubescens* can be distinguished from that of *mexicanus* by the dense pubescence and sparse pilosity. Both workers and females can be separated from those of *interjectus coloradensis* by the sparse rather than numerous erect hairs and dense rather than sparse pubescence. From the other species with very small females, *L. (A.) occidentalis* and *plumopilosus*, it differs in characters too numerous for consideration. *L. (A.) pubescens* can be distinguished from *L. (A.) parvula* M. R. Smith, a small species from Illinois, by its sparse, short pilosity, larger eyes, and dense pubescence.

This species may be parasitic on one of the varieties of *Lasius niger*.

#### *Lasius (Acanthomyops) clavigeroides*, n. sp.

##### WORKER

Length, 3.48–4.02 mm.

Head, excluding the mandibles, as broad as long, with straight or slightly convex posterior border and moderately convex sides. Mandibles 8-toothed, the basal-most tooth pointing posteriorly. Clypeus rounded or feebly subangulate in front. Frontal carinae scarcely diverging behind. Scapes incrassated distally, reaching the posterior lateral corners of the head. Funiculi incrassated, the penultimate joints a little broader than long. Eyes small, hairy, with about 30–35 facets. Maxillary palpi short,

3-jointed. Pro- and mesonotum moderately convex in profile, less so than in *claviger*. Base of epinotum in profile moderately convex, declivity straight; the base about three-fifths as long as the declivity. Petiole smaller and with a blunter superior border than *claviger*, in profile with imperceptibly convex anterior and posterior faces; when seen from behind, the petiole straight or faintly excised above but not notched as in *claviger*.

Entire body strongly shining but not quite as shining as *claviger*, the surface often concealed a little by the pubescence.

Erect hairs sparser, much shorter, and less flexuous than on *claviger*, nearly absent on gula, front, and femora; more abundant on the gaster than on other body surfaces. Pubescence moderately dense on the head, sparse on the thorax, varying from rather sparse to moderately dense on the gaster. Pubescence on all regions usually more profuse than on the corresponding regions of *claviger*.

Color varying from light to brownish yellow, the head never reddish.

#### FEMALE

Length, 5.67–6.23 mm.

Head, excluding the mandibles, insignificantly broader than long, with feebly excised posterior border and slightly convex sides. Mandibles 7-toothed, basal borders edentate. Clypeus subangularly produced in front. Eyes hairy, moderately convex. Scapes thickened distally, not quite reaching the posterior lateral corners of the head. Funiculi clavate, the penultimate joints about 1.4 times as broad as long; the second joint a little less than one-half as broad as the penultimate. Maxillary palpi short, 3-jointed. Thorax with the shape of *claviger*. Petiole small, in profile with flat anterior and posterior surfaces, the superior border blunt; seen from behind, narrow, the sides straight above, the superior border feebly convex, sinuate, or faintly notched. Femora and tibiae slightly flattened, the fore femora about three times, the middle and hind femora about four times, as long as broad. Wings about 6.3 mm. long.

Entire insect shining, but not glabrous like *claviger*, the shining surface somewhat hidden by the pubescence.

Erect hairs sparser, much shorter and less flexuous than on *claviger*, lacking the microscopic plumosity which characterizes the hairs of many *claviger* queens. No hairs on the front, very few or none on the vertex and occiput. Pubescence shorter and much denser than that of *claviger*, particularly on the head and gaster where it is dense enough to give a slight pruinose effect; sparser on the dorsum of the thorax.

Color dark blackish brown, appendages lighter. Veins and stigma of the wings pale brown.

#### MALE

Length, 3.93–4.71 mm.

Head, excluding the mandibles, a little broader than long, with moderately convex posterior border and sides, a little broader behind

than in front. Each mandible with only a strong apical tooth as in *claviger*. Scapes slightly surpassing the posterior lateral corners of the head. Eyes very convex, hairy, situated in the middle of the sides of the head. Maxillary palpi short, 3-jointed. Thorax slightly narrower than the head, not as robust in profile as *claviger*. Petiole small, the superior border not very sharp; in profile, with feebly concave anterior and feebly convex posterior faces. Wings about 4.6 mm. long.

Head and thorax weakly shining, gaster moderately so.

Erect hairs shorter and sparser than on *claviger*. Pubescence rather sparse, but denser than on *claviger*.

Color black; legs and funiculi lighter.

Described from 65 virgin females, 27 males, and 110 workers taken from a sandy, low mound nest in oak woods near Solon Springs, Wis., August 15, 1941.

*L. (A.) clavigeroides* seems to be very closely related to *L. (A.) claviger*. In the field the worker, female, and male appear like small replicas of the corresponding phases of *claviger*. The striking differences in pilosity and pubescence entitle *clavigeroides* to full specific rank, however. The female of *clavigeroides* can be distinguished from that of *claviger* by the very short, sparse pilosity, much denser pubescence, smaller petiole which is blunter above and only minutely notched if at all, less flattened legs, and smaller size. The worker of *clavigeroides* can be separated from that of *claviger* by its smaller size, much sparser, shorter pilosity, somewhat denser pubescence, blunter, unnotched petiole, and less convex sides of the head. *L. (A.) clavigeroides* differs from *claviger subglaber* by most of the above points. In addition the queens differ considerably in color.

*L. (A.) clavigeroides* workers can be distinguished from *L. (A.) parvula* by their larger size, shorter, sparser pilosity and somewhat shorter, more incrassated antennae. Queens and workers of *clavigeroides* and *pubescens* can be readily distinguished by their antennae.

*L. (A.) occidentalis* differs from *clavigeroides* by having the petiole very large, its superior border sharp and emarginate, and by having the head of the female long, rectangular, and narrower than the thorax. The pile is also more abundant than on *clavigeroides*.

This species is probably a temporary social parasite of one of the varieties of *Lasius niger*. However, *L. brevicornis* was also present in the type locality and may possibly act as the host.

**EIMERIA NEOLEPORIS N. SP., OCCURRING NATURALLY IN THE  
COTTONTAIL AND TRANSMISSIBLE TO THE TAME RABBIT<sup>1</sup>**

JOSÉ CANDIDO M. CARVALHO<sup>2</sup>

*From the Section of Entomology and Economic Zoology, Iowa Agricultural Experiment Station*

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During the period from August through December, 1941, microscopic examinations were made of the colic content or feces of 15 cottontails (*Sylvilagus floridanus mearnsii* Allen) taken in the vicinity of Ames, Iowa. Four of the animals were found to harbor oöcysts of a hitherto undescribed species of *Eimeria*. A careful morphological study was made of the fresh, sporulating, and sporulated oöcysts, and the time required for sporulation in a layer of 3 per cent potassium dichromate solution of about 2 mm. depth in Petri dishes at room temperature was observed. Successful attempts were made to infect previously uninfected tame rabbits (*Lepus cuniculus*), so that it was possible to determine the prepatent periods and patent periods for infections in the latter host. The critical characters of the oöcysts have been determined as follows:

***Eimeria neoleporis*, n. sp.**

**Shape:** subcylindrical or elongate ellipsoidal, usually tapering somewhat toward the micropyle.

**Color:** pinkish yellow.

**Micropyle:** present, very distinct (except in certain perhaps abnormal specimens appearing at the end of heavy infections).

**Oöcyst wall:** smooth, same thickness throughout, enlarging noticeably near the micropyle.

**Extra-residual body:** consisting of 4 or less granules at the sporoblast stage, or absent, usually disappearing after completion of sporulation.

**Intra-residual body:** present, large, occupying about  $\frac{1}{3}$  of the sporoblast.

**Protoplasmic contents:** a compact sphere in the center of the oöcyst.

Toward the end of heavy infections usually many oöcysts are eliminated with the contents diffused throughout. Such oöcysts fail to sporulate.

**Sporulation time:** 50 to 75 hours; average 60 hours.

**Length:** 32.8–44.3  $\mu$ , mean, 38.8  $\mu$ ; most frequent, 38.5  $\mu$ .

**Breadth:** 15.7–22.8  $\mu$ , mean, 19.8  $\mu$ ; most frequent, 20.0  $\mu$ .

**Mean shape index:** 1.95.

**Spores:** with Stieda body, elliptical, measuring on the average 17.1  $\mu$  in length by 8.0 to 9.0  $\mu$  in width.

**Sporozoites:** banana-shaped as usual, with nucleus, large and small refractive granules.

<sup>1</sup> Journal paper No. J-976 of the Iowa Agricultural Experiment Station, Ames, Iowa, Project 570. The Fish and Wildlife Service (U. S. Dept. of the Interior), Iowa State Conservation Commission, and American Wildlife Institute cooperating.

<sup>2</sup> Grateful acknowledgment is made of technical assistance by Dr. E. R. Becker.

Polar inclusion of the oöcyst: not observed.

Prepatent period: 11 to 14 days; mean, 12 days.

Patent period: 8 to 16 days; mean, 10 days.

Specific diagnosis: closest to *E. leporis* Nieschulz, 1923,<sup>3</sup> from the intestine of the Alpine hare (*Lepus timidus*) in Europe.

Differs principally in: (1) presence of a very distinct micropyle, (2)

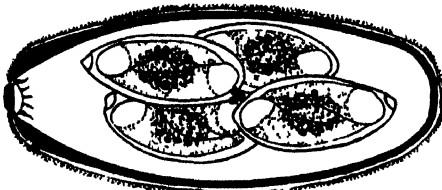


Fig. 1. Sporulated Oöcyst of *Eimeria neoleporis*. x1350.

extra-residual body (when present) of only a few granules, and (3) infectivity to tame rabbit (*Lepus cuniculus*). To date the author has obtained 5 consecutive passages through the latter host and infected in all 26 tame rabbits.

<sup>3</sup> Nieschulz, O. 1923. Über Hasenkokzidien (*Eimeria leporis* n.sp.). Dtsch. tierärztl. Wochenschr., 31: 245-247.





# THE EFFECT OF SODIUM THIOSULFATE AND SODIUM HYDROGEN SULFITE ON SOME SILKS<sup>1</sup>

RUTH L. JOHNSON, MINNIE E. LICHTE, AND RACHEL EDGAR

*From the Sections of Home Economics (Textiles and Clothing) and Chemistry,  
Iowa Agricultural Experiment Station*

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Sodium thiosulfate has been used for the preservation of weighted silks (5, 7) and sodium hydrogen sulfite for the bleaching of silk, the removal of dyes or manganese dioxide from silk, the weighting of silk (2, 10), the preservation of weighted silk (12, 13, 14, 15, and 16), and the printing of dyes on silk (3, 9). Procedures for such treatments, however, occur in the literature without further description of the effect these compounds have on silks.

Quantitative data for the effect of sodium thiosulfate and sodium hydrogen sulfite on some silks are reported in this study. Wild-silk fibroin, silk fibroin, and iron-weighted, lead-weighted, tin-weighted, tin-lead-weighted, and zinc-weighted silks have been analyzed as to weight, ash, nitrogen, and wet strength after 10 hours at 40°C. in 50 volumes of *N* sodium thiosulfate; the unweighted silks have been examined after 10-hour immersion in 50-volume baths of 1.9232 *N* sodium hydrogen sulfite followed by 1 hour's steaming at 123.9°C. These two silks have also been tested for wet strength after 1 hour at 100°C. in 50-volume baths of 0.0500 to 2.0000 *N* sodium hydrogen sulfite.

## EXPERIMENTAL PROCEDURE

The plain-woven silks, described in Table 1 and elsewhere (8), were cut for analysis and the samples were separately treated for 10 hours with 50 volumes of water or *N* sodium thiosulfate in stoppered 500-milliliter Erlenmeyer flasks in a water bath at  $40 \pm 0.1^\circ\text{C}$ . and washed with water until the rinse no longer decolorized permanganate.

The two unweighted silks were immersed separately for 10 hours in 50-volume baths of water or 1.9232 *N* sodium hydrogen sulfite at  $40 \pm 0.1^\circ\text{C}$ ., drained, steamed for 1 hour at 123.9°C., and rinsed with water as described before. Four 4-gram samples of a fabric or five 1-inch strips of it were steamed at a time in an autoclave equipped with pressure gauge and thermometer; the samples were hung by silk thread from glass rods laid across the top of a 4-liter Pyrex beaker having a low outlet for steam and were protected by an inverted watch glass from any liquid which had flowed across metal.

Ten 1-inch strips of an unweighted silk were refluxed on a boiling water bath in 50 volumes of water or sodium hydrogen sulfite for 1 hour

<sup>1</sup>Journal Paper No. J-933 of the Iowa Agricultural Experiment Station, Ames, Iowa. Project 262.

TABLE I  
ANALYSIS OF SILK FABRICS

SILK	WEIGHT OF FABRIC	THICKNESS OF FABRIC	BREAKING STRENGTH OF FABRIC				ELONGATION OF FABRIC AT BREAKING LOAD					
			Conditioned		Wet		Percentage of Conditioned	Percentage of Conditioned	Conditioned		Wet	
			Warp	Filling	Warp	Filling			Warp	Filling	Warp	Filling
	Ounces per Square Yard	0.001 Inch	Pounds per Inch	Pounds per Inch	Warp	Filling	Percentage of Conditioned	Percentage of Conditioned	Warp	Filling	Warp	Filling
A. Iron-weighted.....	3.00 (0.01)*	6.6 (0.1)	34 (1)	12 (0)	97	75	10	7	7	7	25	34
B. Lead-weighted.....	3.17 (0.02)	6.5 (0.2)	30 (0)	12 (1)	57	58	7	7	16	16	23	23
C. Tin-weighted.....	2.86 (0.01)	6.3 (0.3)	38 (1)	13 (1)	53	46	10	9	9	9	35	35
D. Tin-lead-weighted.....	3.15 (0.02)	6.5 (0.2)	42 (0)	13 (1)	57	46	10	7	7	7	35	35
E. Zinc-weighted.....	2.86 (0.01)	6.2 (0.2)	40 (0)	14 (0)	58	50	10	8	8	8	35	31
F. Fibroin.....	2.20 (0.03)	6.7 (0.2)	38 (1)	32 (0)	92	81	34	37	56	56	37	37
G. Wild fibroin.....	1.25 (0.01)	4.2 (0.3)	21 (0)	23 (1)	81	43	30	30	30	30	38	37

\*Mean deviations are within parentheses.

TABLE 1—(Continued)

Srx	DISTRIBUTION OF YARNS IN FABRIC				YARN NUMBER			Twist†	
	By Number		By Weight		Warp	Warp	Filling		
	Warp	Filling	Warp	Filling					
	Number Per Inch	Number Per Inch	Percentage of Fabric	Percentage of Fabric		TypP	TypP		
A. Iron-weighted.....	160 (0)*	74 (0)	69.0 (0.2)	31.1 (0.2)	48.7 (0.3)	46.9 (0.3)	62 (2)		
B. Lead-weighted.....	127 (1)	74 (0)	60.6 (0.2)	37.8 (0.1)	37.4 (0.4)	44.8 (0.2)	31 (2)		
C. Tin-weighted.....	155 (1)	72 (1)	65.1 (0.6)	34.9 (0.6)	44.8 (0.6)	44.8 (0.0)	75 (5)		
D. Tin-lead-weighted.....	180 (1)	79 (1)	71.4 (0.1)	28.5 (0.2)	45.4 (0.3)	48.5 (0.5)	84 (4)		
E. Zinc-weighted.....	170 (0)	70 (1)	73.0 (0.9)	26.0 (1.1)	51.9 (0.4)	48.5 (0.0)	62 (3)		
F. Fibroin.....	246 (2)	109 (1)	59.0 (0.5)	40.3 (0.3)	127.8 (1.8)	88.2 (0.1)	60 (2)		
G. Wild fibroin.....	76 (1)	77 (1)	45.3 (0.7)	53.7 (0.7)	71.7 (0.6)	78.0 (0.5)	1		

\*Mean deviations are within parentheses.

†Two S-twisted crepe yarns alternate with two Z-twisted crepe yarns the length of the warp; the multifilament yarns of the warp and of the wild fibroin are not measurably twisted.

TABLE 1  
ANALYSES OF SILK FABRICS (*continued*)

Silk	ASH			WEIGHTING	WATER EXTRACT
	Fabric	Warp	Filling		
	Percentage of Fabric	Percentage of Yarn	Percentage of Yarn	Percentage of Fabric	Percentage of Fabric
A. Iron-weighted.....	43.70 (0.00)*	42.51 (0.07)	40.23 (0.22)	70.6 (0.7)	10.9 (0.3)
B. Lead-weighted.....	44.16 (0.03)	44.49 (0.28)	44.55 (0.10)	55.3 (0.3)	8.8 (0.5)
C. Tin-weighted.....	52.87 (0.02)	54.22 (0.07)	52.93 (0.04)	61.3 (0.1)	7.2 (0.4)
D. Tin-lead-weighted.....	50.27 (0.06)	52.63 (0.12)	48.72 (0.00)	60.8 (0.6)	4.4 (0.6)
E. Zinc-weighted.....	51.93 (0.08)	53.68 (0.07)	49.93 (0.07)	63.5 (0.4)	4.6 (1.5)
F. Fibroin.....	0.30 (0.03)	.....	.....	.....	.....
G. Wild fibroin.....	0.60 (0.01)	.....	.....	.....	.....

\*Mean deviations are within parentheses.

TABLE 1—(Continued)

SILK	ALUMINUM Percentage of Fabric	IRON Percentage of Fabric	LEAD Percentage of Fabric	NITROGEN Percentage of Fabric	PHOSPHORUS Percentage of Fabric	SILICA Percentage of Fabric	TIN Percentage of Fabric	ZINC Percentage of Fabric
A. Iron-weighted.....	15.11 (0.02)*	.....	12.21 (0.04)	8.16 (0.03)	0.56 (0.00)	12.52 (0.02)	.....	.....
B. Lead-weighted.....	trace	.....	.....	9.17 (0.05)	0.91 (0.01)	20.30 (0.06)	.....	.....
C. Tin-weighted.....	.....	.....	.....	9.26 (0.04)	0.45 (0.01)	20.49 (0.07)	8.80 (0.05)	.....
D. Tin-lead-weighted.....	.....	.....	.....	9.10 (0.03)	8.46 (0.04)	1.06 (0.00)	14.91 (0.08)	8.66 (0.06)
E. Zinc-weighted.....	0.85 (0.02)	.....	.....	.....	8.10 (0.07)	0.57 (0.00)	15.07 (0.06)	.....
F. Fibroin.....	.....	.....	.....	.....	18.59 (0.02)	.....	.....	11.12 (0.02)
G. Wild fibroin.....	.....	.....	.....	.....	18.25 (0.00)	.....	.....	.....

\*Mean deviations are within parentheses.

TABLE 2  
EFFECT OF FIFTY VOLUMES OF SODIUM THIOSULFATE IN TEN HOURS AT 40°C. ON THE WEIGHT, ASH, NITROGEN, AND WET STRENGTH  
OF THE SILK FABRICS

SILK		SODIUM THIOSULFATE	WEIGHT	ASH	NITROGEN	BREAKING STRENGTH OF WET WARP
	Normality	Percentage of Fabric	Percentage of Fabric	Percentage of Fabric	Pounds Per Inch	
A. Iron-weighted.....	0	96.5 (0.1)*	40.23 (0.07)	8.08 (0.03)	32 (2)	
	1	108.7 (0.0)	50.58 (0.17)	8.05 (0.02)	31 (1)	
B. Lead-weighted.....	0	93.0 (0.1)	44.07 (0.07)	9.08 (0.03)	20 (1)	
	1	101.6 (0.1)	44.17 (0.08)	9.04 (0.01)	19 (0)	
C. Tin-weighted.....	0	93.1 (0.3)	51.35 (0.31)	8.64 (0.05)	24 (0)	
	1	99.2 (0.1)	48.60 (0.07)	8.59 (0.05)	20 (0)	
D. Tin-lead-weighted.....	0	96.0 (0.1)	49.04 (0.08)	8.50 (0.04)	21 (1)	
	1	102.2 (0.1)	53.45 (0.10)	8.33 (0.01)	18 (2)	
E. Zinc-weighted.....	0	96.0 (0.1)	50.06 (0.31)	7.79 (0.02)	21 (1)	
	1	94.3 (0.1)	43.28 (0.06)	8.11 (0.07)	18 (1)	
F. Fibroin.....	0	99.8 (0.0)	0.30 (0.01)	18.56 (0.04)	34 (1)	
	1	99.8 (0.0)	0.32 (0.01)	.....	34 (1)	
G. Wild fibroin.....	0	99.7 (0.0)	0.60 (0.01)	18.25 (0.03)	15 (1)	
	1	99.6 (0.1)	0.66 (0.05)	.....	17 (1)	

\*Mean deviations are within parentheses.

TABLE 3  
THE WEIGHT, ASH, NITROGEN, AND WET STRENGTH OF SILK FIBROIN AND WILD SILK FIBROIN AFTER TEN HOURS' IMMERSION AT 40°C. IN  
FIFTY VOLUMES OF SODIUM HYDROGEN SULFITE FOLLOWED BY ONE HOUR'S STEAMING AT 123.9°C.

Silk	SODIUM HYDROGEN SULFITE	STEAMING Hour	WEIGHT	ASH	NITROGEN	BREAKING STRENGTH OF WET WARP	
						Percentage of Fabric	Pounds Per Inch
F. Fibroin.....	0	0	99.8 (0.0)*	0.30 (0.01)	18.56 (0.04)	34 (1)	
	1.9232	0	99.8 (1.0)	0.28 (0.03)	18.48 (0.05)	33 (1)	
	0	1	98.3 (0.4)	0.13 (0.01)	18.45 (0.01)	25 (2)	
	1.9232	1	97.8 (0.2)	0.29 (0.03)	18.45 (0.01)	22 (2)	
	.....	.....	.....	.....	.....	.....	.....
	.....	.....	.....	.....	.....	.....	.....
G. Wild fibroin.....	0	0	99.7 (0.0)	0.60 (0.01)	18.25 (0.03)	15 (1)	
	1.9232	0	99.7 (0.1)	0.65 (0.03)	18.22 (0.02)	16 (1)	
	0	1	98.8 (0.2)	0.20 (0.00)	18.22 (0.02)	14 (1)	
	1.9232	1	97.5 (0.1)	0.76 (0.05)	18.15 (0.05)	14 (1)	
	.....	.....	.....	.....	.....	.....	.....
	.....	.....	.....	.....	.....	.....	.....

\*Mean deviations are within parentheses.

TABLE 4

EFFECT OF FIFTY VOLUMES OF SODIUM HYDROGEN SULFITE IN ONE HOUR AT 100°C. ON THE WET STRENGTH OF SILK FIBROIN AND WILD SILK FIBROIN

SODIUM HYDROGEN SULFITE	BREAKING STRENGTH OF WET WARP	
	Fibroin Pounds per Inch	Wild Fibroin Pounds per Inch
Normality		
0.0000.....	20 (1)*	19 (0)
0.0500.....	.....	18 (1)
0.1000.....	20 (2)	19 (1)
0.2500.....	21 (1)	18 (1)
0.5000.....	.....	19 (0)
1.0000.....	21 (2)	18 (1)
2.0000.....	19 (0)	18 (1)

\*Mean deviations are within parentheses.

and washed with water until the rinse did not decolorize permanganate.

Wet warp strength was determined at once by the inch-strip method (1), total nitrogen after drying the residual silks at room temperature, and ash after drying the silks to constant weight at 105° to 110°C. The total nitrogen of the unweighted silks was determined by the Kjeldahl-Gunning-Arnold method (6) and the total nitrogen of the weighted silks by the Kjeldahl method (11). The fabrics were ashed until constant in an electric furnace at dull red heat. The solutions of sodium thiosulfate were standardized by titration against a standard solution of iodine (4), the solutions of sodium hydrogen sulfite by titration against standard potassium permanganate.

Each value for weight, ash, or nitrogen in Tables 2 and 3 is the mean of four parallel determinations based on the weight of the original fabric dried at 105° to 110°C.; values for wet strength in Tables 2, 3, and 4 are the means of at least ten determinations.

#### SUMMARY

1. Quantitative comparison has been made of the effect of 50-volume baths of water and of  $N$  sodium thiosulfate in 10 hours at 40°C. on the residual weight, ash, nitrogen, and wet strength of plain-woven wild-silk fibroin, silk fibroin, and iron-weighted, lead-weighted, tin-weighted, tin-lead-weighted, and zinc-weighted silks. Similar data have been presented for the effect of 50-volume baths of 1.9232  $N$  sodium hydrogen sulfite in 10 hours at 40°C. and for this treatment followed by 1 hour's steaming at 123.9°C. But slight changes occurred in the total nitrogen or wet strength of the silks during these treatments. The effect of sodium thiosulfate on the weight and ash of the weighted silks appears anomalous.

2. Sodium hydrogen sulfite, in 50-volume baths, 0.0500 to 2.0000  $N$ , has been shown to have no effect in 1 hour at 100°C. on the wet strength of either silk fibroin or wild-silk fibroin.

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# THE $\chi^2$ CORRECTION FOR CONTINUITY<sup>1</sup>

W. G. COCHRAN

*From the Statistical Section, Iowa Agricultural Experiment Station*

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## INTRODUCTION

The correction for continuity, devised by Yates (1, 2), has proved a useful device for extending the use of the  $\chi^2$  test of significance to data in which the expectations are small. For the two cases which he investigated in detail—the binomial distribution and the  $2 \times 2$  contingency table—Yates gave simple rules for applying the correction, and a table of the exact significance levels of the corrected  $\chi^2$ . He also suggested that for contingency tables larger than the  $2 \times 2$  table, there appeared to be less need for a correction for continuity.

From correspondence with several research workers, it appears that there is some uncertainty about the need for a correction for continuity and about the method of making the correction, in cases not explicitly discussed by Yates. The object of this paper is to illustrate the principles involved in correcting for continuity on two of the more common applications of  $\chi^2$ , in the hope of providing a basis for judgment in any particular problem. Some investigation has also been made of the allied problem of the effect of a low expectation at one tail on the  $\chi^2$  test for goodness of fit.

## THE CORRECTION FOR CONTINUITY

While the theoretical basis for the correction has been described by Yates (1) and Fisher (2), a brief outline will be given here for completeness. In all applications of the  $\chi^2$  test to be considered, the exact distribution of  $\chi^2$  is discontinuous, only a finite number of discrete values of  $\chi^2$  being possible. For example, in Figure 1 the exact distribution of  $\chi^2$  has been worked out for a problem (to be discussed later) in which  $\chi^2$  has 10 degrees of freedom. The heights of the vertical lines give the probabilities of all possible values of  $\chi^2$ , which in this case are equally spaced at intervals of  $4/3$ . The sum of these heights is, of course, unity. To calculate the exact probability of a value of  $\chi^2$  as great as or greater than 20, for instance, we add the heights of all lines for which  $\chi^2$  is 20 or greater.

The tabulated distribution of  $\chi^2$ , on the other hand, is a continuous frequency curve,  $\chi^2$  taking all values from zero to infinity. The probability that  $\chi^2$  exceeds a value  $\chi^2_{\alpha}$ , as given in the tables, is the area of the tail of this frequency curve from  $\chi^2_{\alpha}$  to infinity. In using the continuous curve as an approximation to the exact probabilities, each vertical line must be made to correspond to some area under the curve. The most natural

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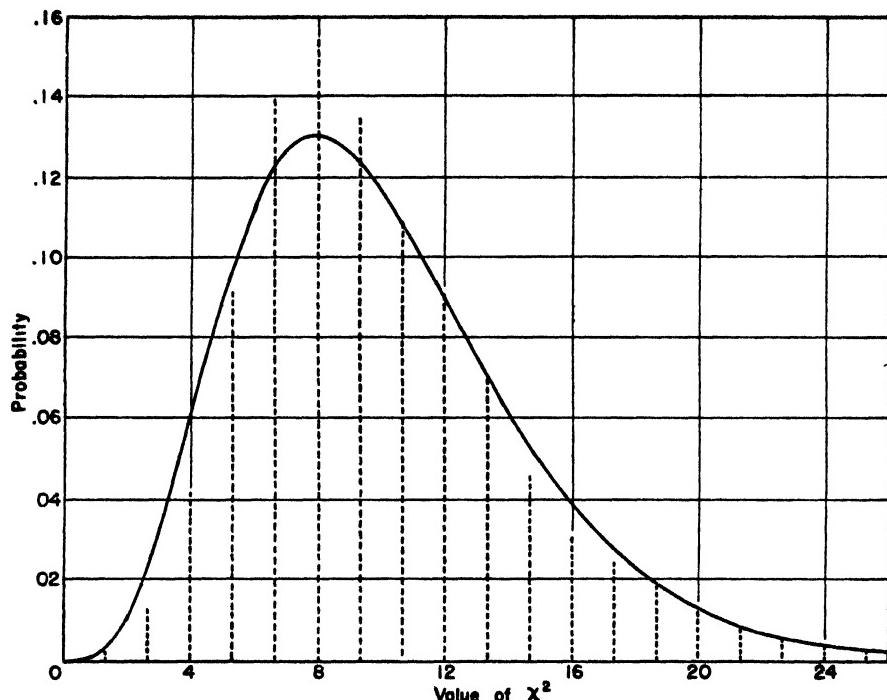


Fig. I—Comparison of Exact and Tabular  $\chi^2$  for 10 Degrees of Freedom

approach is to regard the exact discontinuous distribution as a *grouping* of the tabular distribution, each possible value of the exact  $\chi^2$  representing all values of the continuous  $\chi^2$  which are nearer to it than to any other permissible value of  $\chi^2$ . Thus, the exact value  $\chi^2 = 20$  corresponds to all values of the continuous  $\chi^2$  between  $19\frac{1}{3}$  and  $20\frac{2}{3}$ , since the next lowest value of the exact  $\chi^2$  is  $18\frac{2}{3}$ , while the next highest is  $21\frac{1}{3}$ . Consequently, the exact probability of a value of  $\chi^2$  equal to 20 is approximated by the area under the tabulated distribution from  $\chi^2 = 19\frac{1}{3}$  to  $\chi^2 = 20\frac{2}{3}$ .

It follows that the probability of a value of  $\chi^2$  as great as or greater than 20 is estimated by the area under the curve from  $\chi^2 = 19\frac{1}{3}$  to infinity, not from  $\chi^2 = 20$  to infinity. In this example the exact probability is .0354, while the tabular probabilities are .0362 for  $\chi^2 = 19\frac{1}{3}$  and .0292 for  $\chi^2 = 20$ , showing a marked improvement by correcting for continuity. Similarly, the probability that  $\chi^2$  is less than or equal to 20 is estimated by the area under the curve from zero to  $20\frac{2}{3}$ , i.e., by taking one minus the tabular probability for  $\chi^2 = 20\frac{2}{3}$ . To estimate the probability that  $\chi^2$  lies between 12 and 20 inclusive, we would calculate the area of the curve between  $11\frac{1}{3}$  and  $20\frac{2}{3}$ .

For most tests of significance the probability wanted is that of a value of  $\chi^2$  as great as or greater than the observed  $\chi^2$ . For this purpose, the general rule in applying corrections for continuity may be stated as fol-

lows: Calculate  $\chi^2$  by the usual formula. Find the next lowest possible value of  $\chi^2$  to the one to be tested, and use the tabular probability for a value of  $\chi^2$  midway between the two.

If the possible values of  $\chi^2$  are closely spaced together, the probabilities given by the uncorrected  $\chi^2$  and the corrected  $\chi^2$  may differ only by an amount that is regarded as negligible. In this case the correction may be ignored.

The rule given by Yates for correcting for continuity is to decrease the absolute difference between the observed and the expected values by one-half. It is important to trace the relation between this rule and the general rule given above. For the two cases discussed by Yates (1)—the binomial and the  $2 \times 2$  contingency table— $\chi^2$  has only a single degree of freedom, the difference between the observed value  $x$  and the expected value  $m$  being the same for all cells. Thus,  $\chi^2$  is of the form  $(x - m)^2$

$(\sum \frac{1}{m})$ , the summation being over all cells, while  $\chi$  may be written

$(x - m) \sqrt{\sum \frac{1}{m}}$ . Yates actually dealt with the frequency distribution

of  $\chi$ , for which the tabular value follows the normal law. The advantage in so doing is that a distinction may be drawn between positive and negative values of  $(x - m)$ , this procedure being convenient when it is desired to test the deviation in one direction only, or when the exact distribution of  $\chi$  is skew. Thus, Yates was concerned with the problem of estimating the probability at one tail of the distribution of  $\chi$ . Since  $x$  takes only a succession of integral values, successive values of  $(x - m)$  must differ by unity. Hence, to correct  $\chi$  for continuity by the general rule, the difference between the observed and expected values should be reduced in absolute magnitude by one-half, as Yates proposed. The point to be stressed here, however, is that this rule should be used only when it agrees with the general rule given previously.

#### TESTS FOR LINKAGE

A convenient example for further illustration of the principles is provided by the use of  $\chi^2$  in testing for linkage between two factors in genetical studies. If doubly heterozygous individuals  $AaBb$  are inbred, where  $A$  and  $B$  are dominant, the expectations of the four phenotypes  $AB$ ,  $Ab$ ,  $aB$  and  $ab$  in the  $F_2$  generation are as  $9 : 3 : 3 : 1$ , respectively. The results of such an experiment may therefore be put in the form:

TYPE	$AB$	$Ab$	$aB$	$ab$	TOTAL
Number observed.....	$a$	$b$	$c$	$d$	$n$
Number expected.....	$\frac{9n}{16}$	$\frac{3n}{16}$	$\frac{3n}{16}$	$\frac{n}{16}$	$n$

It is easily seen that  $\chi^2$ , with 3 degrees of freedom, may be written as

$$\chi^2 = \frac{16}{n} \left( \frac{a^2}{9} + \frac{b^2}{3} + \frac{c^2}{3} + \frac{d^2}{1} \right) - n$$

As Fisher (2, § 51) has shown, this quantity may be divided into three single-degrees of freedom, as follows.

$$\chi^2_1 = (a+b-3c-3d)^2/3n$$

$$\chi^2_2 = (a+c-3b-3d)^2/3n$$

$$\chi^2_3 = (a+9d-3b-3c)^2/9n$$

The quantities  $\chi^2_1$  and  $\chi^2_2$  test whether the observations are segregating in a 3 : 1 ratio for the *A* and *B* factors, respectively. These are simply tests of a single binomial ratio, and are completely covered by Yates's discussion (1). To correct  $\chi^2_1$  and  $\chi^2_2$  for continuity in the above form, the quantity inside the bracket should be reduced by two in absolute magnitude,<sup>2</sup> since, for fixed *n*,  $(a+b-3c-3d)$  and  $(a+c-3b-3d)$  change by intervals of four.

The quantity  $\chi^2_3$  serves as a test for linkage between the two characters. For fixed *n*, the smallest change in  $(a+9d-3b-3c)$  is also  $\pm 4$ , produced by decreasing *a* and increasing *b* or *c* by unity. Hence, to correct  $\chi^2_3$  for continuity,  $(a+9d-3b-3c)$  should also be reduced by two in absolute magnitude. The effect of the correction is smaller with  $\chi^2_3$  than with  $\chi^2_1$  or  $\chi^2_2$ , owing to the larger divisor,  $9n$ . Since the difference between the uncorrected and the corrected value of  $\chi^2_3$  is  $2/3\sqrt{n}$ , it is easy to calculate a value of *n* above which correction is unnecessary. If the uncorrected value is to underestimate the probability by not more than 0.5 per cent at the 5 per cent level, *n* should be at least 300. It should be noted that  $\chi^2_3$  cannot be interpreted as the test of a single binomial ratio, so that Yates's table of the exact significance levels of the corrected  $\chi^2$  does not apply to this case.

Since the individual degrees of freedom provide tests of different aspects of the data, there is probably little interest in testing the combined  $\chi^2$  (with three degrees of freedom), except perhaps as a preliminary indication. To correct the combined  $\chi^2$ , the general rule must be applied, by finding the next lowest possible value of  $\chi^2$ . No simple arithmetical rule appears to be available for doing this. The minimum possible change in the quantity  $(a^2+3b^2+3c^2+9d^2)$  is  $\pm 2$ , so that the corresponding change in the combined  $\chi^2$  is  $\pm 32/9n$ . There may, however, be gaps in the succession of values of  $\chi^2$ , and each case requires special inspection. An example will illustrate the relation between the corrections of the individual degrees of freedom and of the combined  $\chi^2$ . The data are taken from an experiment by Imai (3), quoted by Mather (4), the values being *a*=47, *b*=8, *c*=11, *d*=9.

The quantities  $\chi^2_1$ ,  $\chi^2_2$  and  $\chi^2_3$  are corrected by the rule already given.

<sup>2</sup>B. L. Wade, Unpublished typescript.

TABLE 1  
CORRECTION FOR CONTINUITY IN A LINKAGE TEST

	VALUE OF $\chi^2$		PROBABILITY		PERCENTAGE ERROR IN UNCORRECTED PROBABILITY
	Uncorrected	Corrected	Uncorrected	Corrected	
$\chi_1^2$ .....	0.111	0.040	.739	.841	-13
$\chi_2^2$ .....	0.218	0.111	.640	.739	-13
$\chi_3^2$ .....	7.468	7.053	.0063	.0079	-21
Combined $\chi^2$ ....	7.797	7.750	.0505	.0515	- 2

By trial, the next lowest value of the combined  $\chi^2$  appears to be 7.702, given by the configuration  $a=45$ ,  $b=7$ ,  $c=14$ ,  $d=9$ . Hence, the corrected value of the combined  $\chi^2$  is  $1/2(7.797+7.702)=7.750$ . It should be noted that the corrected values of  $\chi^2$  are not additive, the total of the corrected single degrees of freedom being 7.204, while the combined value, after correction, is 7.750. Further, the effect of correction is much smaller on the combined  $\chi^2$  than on the single degrees of freedom. The difference between the probabilities given by the uncorrected and corrected values of the combined  $\chi^2$  is negligible.

#### THE ADDITION OF SINGLE DEGREES OF FREEDOM

A further example, also common in genetical work, arises when observations on the number of individuals of each of two types,  $A$  and  $a$ , are available for several different families. For each family we may test whether the individuals are segregating in a given ratio, say 3:1, by calculating the appropriate  $\chi^2$ , as described in the previous section. If all families are presumed to be segregating in the same ratio, it is customary to add the individual values of  $\chi^2$  to form a "total"  $\chi^2$ . This is divided into two parts:  $\chi_1^2$ , (with 1 degree of freedom) which tests whether the totals of the  $A$ 's and  $a$ 's over all families are segregating in the proposed ratio, and  $\chi_{1n}^2$ , found by subtracting  $\chi_1^2$  from the "total"  $\chi^2$ . Of these,  $\chi_1^2$  provides a sensitive test for any *consistent* departure of the families as a whole from the proposed ratio, while  $\chi_{1n}^2$  (sometimes called the "heterogeneity"  $\chi^2$ ) tests whether the families agree mutually in their segregation ratio. The use of these tests is, of course, not confined to genetical investigations.

When testing any individual family, the  $\chi^2$  should of course be corrected for continuity. The question arises: Should corrected or uncorrected values of  $\chi^2$  be added when forming the "total"  $\chi^2$ ? It is clear from the discussion in the previous section that the uncorrected values must be used when computing the "total"  $\chi^2$ , which should then be corrected, if necessary, by the general rule given. As a demonstration, we have worked out for an extreme case the exact distribution of the "total"  $\chi^2$  as

computed in each of the two proposed ways. The data consist of ten families, each containing only four observations, segregating 3 to 1. Thus, the "total"  $\chi^2$  has ten degrees of freedom,

Since there are only four observations per family,  $a$  and  $A$  can take only the values 0, 1, 2, 3, and 4, with probabilities which are given by the binomial  $\left(\frac{3}{4} + \frac{1}{4}\right)^4$ . All possible results for a single family are shown in Table 2 below, with the corresponding probabilities and values of  $\chi^2$  with and without correction for continuity.

TABLE 2  
POSSIBLE VALUES OF  $\chi^2$  FOR FOUR OBSERVATIONS, SEGREGATING 1:3

$a$	$A$	PROBABILITY	VALUE OF $\chi^2$	
			Uncorrected	Corrected
0.....	4	81	4/3	1/3
1.....	3	108	0	0
2.....	2	54	4/3	1/3
3.....	1	12	16/3	3
4.....	0	1	12	25/3
		+ 256		

For  $a=0$ ,  $A=4$ , for instance, the value of  $\chi^2$  is, of course,  $(0-1)^2/1 + (4-3)^2/3 = 4/3$ , while the corresponding value, corrected by Yates's rule, is  $(1/2)^2/1 + (1/2)^2/3 = 1/3$ .

The probabilities were computed for all possible combinations of these results from ten families, and also the corresponding values of  $\chi^2$  (with ten degrees of freedom) found by adding the corrected or uncorrected individual  $\chi^2$ 's.

In Table 3, the exact significance levels of the total of the "corrected"

TABLE 3  
COMPARISON OF EXACT AND TABULAR  $\chi^2$  DISTRIBUTIONS, FOR THE TOTAL OF THE  
"CORRECTED" SINGLE DEGREES OF FREEDOM

$\chi^2$	Exact $P \geq \chi^2$	Tabular $P \geq \chi^2$
1.....	.9850	.9998
2.....	.7143	.9963
3.....	.4211	.9814
4.....	.3926	.9473
5.....	.2227	.8912
6.....	.1114	.8153
7.....	.1075	.7254
8.....	.0627	.6288
9.....	.0475	.5321
10.....	.0375	.4405
11.....	.0167	.3575

$\chi^2$ 's are compared with these given by the tables. The possible values of the total of the corrected  $\chi^2$ 's are spaced at intervals of one-third. To save space only every third value is shown below.

The tabular  $\chi^2$  overestimates the probabilities so violently that there can scarcely be said to be any relation between the two distributions. For instance, ten is the mean value of the tabular  $\chi^2$ , yet a value of ten or more is reached only about once in thirty times by the total of the "corrected"  $\chi^2$ 's. The use of the  $\chi^2$  table would lead very frequently to the apparent conclusion that the data were too homogeneous. If the tabular  $\chi^2$  were corrected for continuity, the agreement would be still worse.

The exact distribution of the total of the uncorrected values of  $\chi^2$  is compared with the corresponding tabular  $\chi^2$  in Figure 1 above.<sup>3</sup> The agreement appears remarkably good, at least in the upper tail which is relevant to tests of significance. A more exact comparison in the region between .10 and .01 is shown in Table 4.

TABLE 4  
COMPARISON OF EXACT AND TABULAR  $\chi^2$  DISTRIBUTIONS, FOR THE TOTAL OF THE  
"UNCORRECTED" SINGLE DEGREES OF FREEDOM

$\chi^2$	Exact $P \geq \chi^2$	Tabular $P \geq \chi^2$	Tabular, Corrected for Continuity
16.00.....	.1076	.0996	.1204
17.33.....	.0773	.0673	.0821
18.67.....	.0534	.0447	.0550
20.00.....	.0354	.0292	.0362
21.33.....	.0236	.0189	.0235
22.67.....	.0158	.0120	.0151
24.00.....	.0102	.0076	.0096

Since, owing to the small number of observations, the successive values of  $\chi^2$  are rather far apart, the tabular  $\chi^2$  requires correction for continuity, as shown in the right-hand column. The agreement with the exact probabilities in the critical region is excellent.

A similar comparison for the "heterogeneity"  $\chi^2$  would show approximately the same results. Thus, the correct procedure is to form the "total"  $\chi^2$  by adding the uncorrected single degrees of freedom. To form  $\chi^2_A$ , subtract  $\chi^2_t$ , calculated without correction. In testing  $\chi^2_A$ , however, a correction should be made, since we are testing a single binomial ratio. Theoretically,  $\chi^2_A$  may also require correction after it has been computed as described above. While there appears to be no simple arithmetical rule for making this correction, its effect should be negligible unless the number of families and the expectations of the two types  $a$  and  $A$  are both small.

It may seem paradoxical that the uncorrected  $\chi^2$ , which underestimates the true probabilities with a single degree of freedom, should give good agreement when several different values are added together, while the corrected  $\chi^2$  behaves in the opposite manner. The reason may be

<sup>3</sup> Since the interval between successive values of  $\chi^2$  is  $4/3$ , the ordinates of the tabular curve have been multiplied by  $4/3$ .

exhibited more clearly by examining the two quantities further. For a single degree of freedom, the exact mean of the uncorrected  $\chi^2$  is 1, while

the variance is  $2(1 + \frac{1-6pq}{2npq})$  where  $n$  is the number of observations and

$p : q$  is the segregation ratio. For the total  $\chi^2$  from  $r$  different families, the corresponding values are, therefore,

$$\text{Mean} = r. \quad \text{Variance} = 2r(1 + \frac{1-6pq}{2n'pq})$$

where  $\frac{1}{n'} = \frac{1}{r} \left( \sum_{i=1}^r \frac{1}{n_i} \right)$  is the average value of  $\frac{1}{n}$  for the different fam-

ilies. The corresponding mean and variance for the tabular  $\chi^2$  are  $r$  and  $2r$ , respectively. Hence, the exact and tabular  $\chi^2$  have always the same mean value, and agree closely in their variances provided that  $(npq)$  is reasonably large. As  $r$  increases, both distributions tend to normality, being determined more and more exactly by the values of their mean and variance. Moreover, as  $r$  increases, the number of possible values of the exact  $\chi^2$  increases rapidly, particularly so if  $n$  varies from family to family, so that the effect of a continuity correction diminishes. Hence, the agreement between the exact and tabular  $\chi^2$  tends to improve as  $r$  increases.

For a single degree of freedom, the correction for continuity reduces the value of  $\chi^2$  in order that the tabular probability may give a better approximation to the true probability at the upper tail. The effect is, however, to reduce both the mean and variance of the corrected  $\chi^2$  below those of the uncorrected  $\chi^2$ , considerably so if  $n$  is small. If the mean of the corrected  $\chi^2$ , for a single degree of freedom, is  $\lambda$ , where  $\lambda$  is less than unity, the mean of the total of  $r$  values is  $\lambda r$ . Thus, the negative bias in the mean as compared with the tabular  $\chi^2$ , is  $r(1-\lambda)$ , which increases steadily as  $r$  increases. Hence, the agreement with the tabular  $\chi^2$  gets steadily worse as the number of families increases.

With a large number of families the distribution of the "heterogeneity"  $\chi^2$ , calculated without correction, also tends to normality with a mean value  $(r-1)$  and a variance which is approximately equal to  $2(r-1)$

$(1 + \frac{1-6pq}{2n'pq})$ , where  $\frac{1}{n'} = \frac{1}{r} \sum \frac{1}{n_i}$ . Since the discrepancy in the vari-

ance does not tend to decrease as  $r$  increases, it would be somewhat more accurate, when the number of families is large, to regard  $\chi^2$  as normally distributed with the above mean and variance than to use the tabular  $\chi^2$ . However, the latter is adequate for most cases arising in practice unless  $p$  or  $q$  is close to unity. If the tabular  $\chi^2$  and the normal approximation are to give probabilities which agree within 0.5 per cent at the 5 per cent level, it may be shown that the average number of observations per family need only exceed 10 if  $p$  is  $1/4$ , but should exceed 140 if  $p$  is  $1/16$ , and 740 if  $p$  is  $1/64$ .

## THE EFFECT OF A SMALL EXPECTATION ON "GOODNESS OF FIT" TESTS

In using  $\chi^2$  to test goodness of fit, the expectations usually become small towards one or both ends of the frequency distribution. As a working rule, it is customary to combine several classes at the ends, if necessary, so that no expectation is less than a conventional minimum, for which Fisher (2) suggests the value 5 and Aitken (5) the value 10. The reasons for this rule are to decrease the discontinuity in the exact  $\chi^2$  distribution and to approximate more closely to the assumptions underlying the tabular  $\chi^2$  distribution, which postulates that the observed values are normally distributed about the expected values. Of these, the second reason is probably the more important, for if the expectations in the central classes are reasonably high, the total number of values of the exact  $\chi^2$  will be large even though the expectations at the tails are small.

In cases where the observed and the fitted distribution disagree most markedly at the tails, this grouping of classes diminishes the sensitiveness of the  $\chi^2$  test, so that it is sometimes more appropriate to calculate  $\chi^2$  without grouping the tails. Since the effects of a low expectation at one tail do not appear to have been examined, the exact distributions of  $\chi^2$  were worked out for three related examples, each having three classes and a total of 20 observations. The expectations in the individual classes were as follows:

	EXPECTATIONS		
	$m_1$	$m_2$	$m_3$
Example 1	11.4	7.6	1.0
Example 2	11.7	7.8	0.5
Example 3	11.94	7.96	0.1

The smallest expectation varies from 1.0 to 0.1, the other two expectations being kept in the ratio 3/2 in all three examples. Since  $m_1$  and  $m_2$  vary little between the three examples, the differences between the resulting  $\chi^2$  distributions should be due mainly to the smallest expectation, which in all cases is well below the usually accepted minimum. The value of  $\chi^2$  is,

of course,  $\sum_{i=1}^3 \frac{(x_i - m_i)^2}{m_i}$ , where  $x_i$  is the observed value in the  $i$ th cell, and

has two degrees of freedom.

For each example, the exact significance levels and the corresponding probabilities given by the tabular  $\chi^2$  (without correction for continuity) are shown for all values of  $\chi^2$  giving probabilities between 0.1 and 0.01.

The examples bring out some interesting points. Except for the extreme case in which the smallest expectation is only 0.1, the successive values of  $\chi^2$  are sufficiently close together so that discontinuity would not of itself introduce gross errors in the probabilities. Further, the exact and tabular probabilities agree reasonably well down to a certain significance level, this being .0271 for  $m_3 = 1$ , .0285 for  $m_3 = 0.5$ , and .1926 for  $m_3 = 0.1$ . Thereafter, the tabular  $\chi^2$  begins rather abruptly to underestimate ser-

TABLE 5  
COMPARISON OF EXACT AND TABULAR  $\chi^2$  IN A GOODNESS OF FIT TEST

SMALLEST EXPECTATION ( $m_3$ )								
1.0			0.5			0.1		
$\chi^2$	Exact $P(\geq \chi^2)$	Tabular $P(\geq \chi^2)$	$\chi^2$	Exact $P(\geq \chi^2)$	Tabular $P(\geq \chi^2)$	$\chi^2$	Exact $P(\geq \chi^2)$	Tabular $P(\geq \chi^2)$
4.561	.1051	.1022	4.752	.1070	.0929	1.985	.3244	.3706
4.640	.0798	.0983	4.923	.0939	.0853	3.451	.1926	.1781
4.921	.0732	.0854	5.034	.0814	.0807	5.335	.1289	.0694
5.272	.0603	.0716	5.308	.0760	.0704	7.638	.1045	.0243
5.930	.0540	.0516	5.649	.0664	.0593	8.174	.0973	.0168
6.105	.0492	.0472	5.855	.0577	.0535	8.216	.0810	.0164
6.395	.0465	.0409	6.290	.0415	.0431	8.551	.0647	.0139
6.456	.0433	.0396	6.744	.0357	.0343	8.677	.0513	.0131
6.535	.0399	.0381	7.214	.0285	.0271	9.347	.0381	.0093
6.877	.0302	.0321	7.701	.0270	.0213	9.556	.0293	.0084
6.982	.0285	.0305	8.205	.0242	.0165	10.360	.0208	.0056
7.377	.0271	.0250	8.385	.0192	.0151	10.561	.0192	.0051
8.079	.0251	.0176	9.265	.0176	.0098	10.854	.0143	.0044
8.430	.0237	.0148	9.539	.0168	.0085	12.194	.0101	.0023
8.947	.0229	.0114	9.820	.0158	.0074			
8.982	.0200	.0112	10.393	.0155	.0055			
9.263	.0191	.0097	10.983	.0150	.0041			
9.483	.0185	.0087	11.804	.0139	.0027			
9.509	.0156	.0086	12.214	.0136	.0022			
9.552	.0156	.0084	12.829	.0134	.0016			
9.553	.0131	.0084	12.855	.0111	.0016			
9.903	.0128	.0071	13.128	.0111	.0014			
10.035	.0106	.0066						

iously. Since only a part of the underestimation in this region would be recovered by correction for continuity, the discontinuity is evidently not the principal cause of the discrepancy. For  $m_3 = 0.1$  the tabular  $\chi^2$  is useless as an approximation throughout the whole of the region between  $p = .1$  and  $p = .01$ . For  $m_3 = 0.5$ , the tabular probability agrees almost as well as for  $m_3 = 1.0$  down to  $p = .028$ , but thereafter the underestimation is more serious, so that at  $p = .01$  the tabular  $\chi^2$  is worse for  $m_3 = 0.5$  than for  $m_3 = 0.1$ .

To investigate these results in more detail, it is necessary to isolate the contribution to  $\chi^2$  from the cell with the small expectation. Let  $n$  be the number of observations and  $p_1, p_2, \dots, p_r$  the probabilities in the  $r$  classes. The observed number  $k$  in the  $r$ th class is distributed in the binomial series  $(q_r + p_r)^n$  where  $q_r = 1 - p_r$ . For a fixed  $k$ , the observed numbers in the remaining classes are distributed in the multinomial

$$\left( \frac{p_1}{q_r} + \frac{p_2}{q_r} + \dots + \frac{p_{r-1}}{q_r} \right)^{n-k}.$$

Consequently, if the expected values in the first  $(r-1)$  classes are sufficiently large, the quantity

$$\chi^2_{r-2} = \frac{q_r}{(n-k)} \sum_{i=1}^{r-1} \left( \frac{k^2_i}{p_i} \right) - (n-k) \quad (1)$$

is distributed as  $\chi^2$  with  $(r-2)$  degrees of freedom. The value of  $\chi^2$  for all classes may be written

$$\chi^2 = \sum_{i=1}^r \frac{k^2_i}{(np_i)} - n \quad (2)$$

Using (1) this becomes, after some algebraic manipulations

$$\chi^2 = \frac{(n-k)}{nq_r} \chi^2_{r-2} + \frac{(k-np_r)^2}{np_r q_r} \quad (3)$$

The first term on the right-hand side of (3) represents the combined contribution to  $\chi^2$  from the first  $(r-1)$  classes, while the second term is the contribution from the  $r$ th class, in which the expectation  $np_r$  will be assumed small.

For a given value of  $k$ , the probability that  $\chi^2$  exceeds any specified quantity depends on the value of  $\chi^2_{r-2}$ , and is easily found from the tabular  $\chi^2$  for  $(r-2)$  degrees of freedom. Since the probabilities of different values of  $k$  are known from the binomial  $(q_r+p_r)^n$ , the total probability that  $\chi^2$  exceeds any specified quantity may be found by adding the probabilities for different values of  $k$ . The significance levels of  $\chi^2$ , as defined by equation (3), clearly depend on three variables: the number of classes,  $r$ ; the smallest expectation,  $np_r$ ; and the total number of observations,  $n$ . However, for fixed  $r$  and  $np_r$ , the significance levels vary little with  $n$ , provided that  $n$  exceeds 20. Since in any case  $n$  must exceed 20 to satisfy the condition that the expectations  $np_1, \dots, np_{r-1}$  are reasonably large, the limiting value as  $n$  tends to infinity was substituted in (3), giving

$$\chi^2_m = \chi^2_{r-2} + \frac{(k-m_r)^2}{m_r} \quad (4)$$

where  $k$  now follows a Poisson distribution with mean  $m_r$ . The symbol  $\chi^2_m$  is used to denote the fact that the distribution depends on the expectation  $m_r$  in the smallest class.

To verify this theoretical approach, the significance levels of  $\chi^2_m$  given by equation (4) were compared with the exact significance levels of  $\chi^2$  for the most extreme example  $m_3=0.1$ .

Since the distribution of  $\chi^2_m$  is continuous, and since the exact values of  $\chi^2$  are somewhat widely spaced, the significance levels of  $\chi^2_m$  are shown both with and without correction for continuity. Before correction, the probabilities are slightly underestimated by  $\chi^2_m$ , but the underestimation is mainly due to the discontinuity in the exact  $\chi^2$ . After correction, the  $\chi^2_m$  probabilities give a satisfactory approximation to the correct probabilities, whereas it will be recalled that the tabular  $\chi^2$  probabilities were

TABLE 6  
COMPARISON OF THE EXACT  $\chi^2$  DISTRIBUTION AND THE  $\chi^2_m$  DISTRIBUTION  
Expectations: 11.94, 7.96, 0.1

$\chi^2$	EXACT $P(\geq \chi^2)$	$P(\geq \chi^2)$ GIVEN BY $\chi^2_m$	
		Uncorrected	Corrected
7.638	.1045	.1006	.1056
8.174	.0973	.0798	.0999
8.216	.0810	.0756	.0773
8.551	.0647	.0534	.0621
8.677	.0513	.0482	.0509
9.347	.0381	.0309	.0380
9.556	.0293	.0272	.0289
10.360	.0208	.0180	.0218
10.561	.0192	.0163	.0171
10.854	.0143	.0144	.0153
12.194	.0101	.0093	.0111

greatly in error. The  $\chi^2_m$  approximation is also sufficiently accurate for practical purposes in the other two examples.

TABLE 7  
PROBABILITY LEVELS OF  $\chi^2_m$  ASSOCIATED WITH THE 5 AND 1 PER CENT LEVELS OF  $\chi^2$

SMALLEST EXPECTATION $m$ .	NUMBER OF DEGREES OF FREEDOM IN $\chi^2$							
	2	3	4	5	6	10	15	25
	5 Per Cent Level							
0.1 . . . . .	.1089	.1142	.0909	.0800	.0741	.0646	.0605	.0576
0.5 . . . . .	.0486	.0522	.0542	.0555	.0564	.0552	.0539	.0528
1.0 . . . . .	.0480	.0537	.0553	.0540	.0534	.0527	.0522	.0516
2.0 . . . . .	.0474	.0527	.0517	.0516	.0518	.0515	.0512	.0509
3.0 . . . . .	.0471	.0507	.0510	.0512	.0512	.0510	.0507	.0507
5.0 . . . . .	.0476	.0499	.0504	.0506	.0507	.0506	.0505	.0504
1 Per Cent Level								
0.1 . . . . .	.0334	.0259	.0229	.0213	.0203	.0180	.0169	.0159
0.5 . . . . .	.0195	.0209	.0197	.0172	.0159	.0139	.0129	.0119
1.0 . . . . .	.0181	.0144	.0139	.0137	.0136	.0123	.0117	.0111
2.0 . . . . .	.0126	.0130	.0128	.0122	.0119	.0117	.0109	.0106
3.0 . . . . .	.0119	.0123	.0118	.0115	.0115	.0110	.0107	.0104
5.0 . . . . .	.0119	.0112	.0112	.0110	.0109	.0106	.0104	.0102

Assuming that the  $\chi^2_m$  distribution takes proper account of the effect of a single small expectation  $m$ , we may estimate the error involved in using the tabular  $\chi^2$  distribution in such cases. The error will depend on the number of degrees of freedom in  $\chi^2$ , since a single small class presumably contributes to a lesser degree when the number of classes is large. For  $m=0.1, 0.5, 1, 2, 3$ , and  $5$  and  $2, 3, 4, 5, 6, 10, 15$ , and  $25$  degrees of freedom in  $\chi^2$ , the probability level of  $\chi^2_m$  are shown below at the  $5$  and  $1$  per cent values of the tabular  $\chi^2$ . These probabilities are to be regarded as more nearly the true probabilities corresponding to apparent probabilities of  $5$  and  $1$  per cent found by using the tabular  $\chi^2$ .

As has generally been supposed, the tabular  $\chi^2$  underestimates the probabilities, except at the  $5$  per cent level with two degrees of freedom (three classes). The error diminishes as the smallest expectation increases and also as the number of degrees of freedom increases, though the rate of improvement is somewhat irregular in certain parts of the table, as might be expected from the nature of the distribution. Except for the most extreme case,  $m=0.1$ , the errors are not alarmingly great. If a  $20$ -per cent error is permitted in the estimation of the true probability (i.e., an error up to  $1$  per cent at the  $5$  per cent level and  $0.2$  per cent at the  $1$  per cent level), the tabular  $\chi^2$  is sufficiently accurate at the  $5$  per cent level for all values of  $m$  down to  $0.5$ . To reach this standard of accuracy for  $m=0.1$ , the number of degrees of freedom in  $\chi^2$  should exceed  $15$ . At the  $1$  per cent level, agreement is not so good. The necessary minimum values of the number of degrees of freedom are shown below for the various values of  $m$ .

Smallest expectation $m$	0.1	0.5	1.0	2.0	3.0	5.0
Minimum number of degrees of freedom	?	25	10	6	4	2

For example, with an expectation of two in the smallest class, the expectations in the other classes being supposed large,  $\chi^2$  must have at least six degrees of freedom if the error at the  $1$  per cent level is to be less than  $0.2$  per cent.

From the above investigation it appears that, with only a single small expectation, the conventional procedure of grouping a class with a lower limit of under  $5$  is on the conservative side. At the  $5$  per cent level, the lower limit could be set as small as  $0.5$ , and at the  $1$  per cent level as low as  $2$  without undue error, though it should be remembered that the error is consistently in the same direction. For those desiring a better approximation to the true probabilities, a small table of the  $5$  and  $1$  per cent significance levels of  $\chi^2_m$  is given in Table 8. These significance levels may be used instead of the tabular  $\chi^2$  where it is desired to avoid grouping a class with an expectation of below  $5$ . The value of  $\chi^2$  is, of course, computed in the usual manner.

For values of  $m$  and numbers of degrees of freedom not shown in the

TABLE 8  
FIVE AND 1 PER CENT SIGNIFICANCE LEVELS OF  $\chi^2_m$

SMALLEST EXPECTATION	NUMBER OF DEGREES OF FREEDOM IN $\chi^2$							
	2	3	4	5	6	10	15	25
	5 Per Cent Level							
0.1.....	8.63	9.82	11.14	12.50	13.87	19.28	25.82	38.37
0.5.....	5.93	7.94	9.74	11.41	13.02	18.67	25.30	37.91
1.....	5.88	8.04	9.79	11.29	12.79	18.49	25.16	37.80
2.....	5.87	7.98	9.57	11.17	12.69	18.41	25.09	37.73
3.....	5.87	7.85	9.54	11.13	12.66	18.37	25.06	37.70
4.....	6.04	7.80	9.53	11.11	12.64	18.36	25.04	37.69
5.....	5.88	7.81	9.51	11.10	12.63	18.35	25.03	37.68
$\infty^*$ .....	5.99	7.82	9.49	11.07	12.59	18.31	25.00	37.65
	1 Per Cent Level							
0.1.....	11.85	14.10	16.04	17.84	19.56	25.94	33.34	47.19
0.5.....	12.78	13.86	15.25	16.78	18.33	24.40	31.59	45.08
1.....	10.31	12.39	14.34	16.17	17.80	23.91	31.13	44.72
2.....	9.85	12.22	13.94	15.64	17.32	23.59	30.88	44.53
3.....	9.64	12.00	13.71	15.47	17.17	23.47	30.78	44.46
4.....	9.56	11.75	13.62	15.38	17.09	23.41	30.73	44.42
5.....	9.82	11.63	13.56	15.33	17.04	23.37	30.70	44.40
$\infty^*$ .....	9.21	11.34	13.28	15.09	16.81	23.21	30.58	44.31

\*Tabular  $\chi^2$  significance levels.

table, interpolation will be necessary. Where the number of degrees of freedom is tabulated, linear interpolation for  $m$  will probably suffice down to  $m=1$ , though harmonic interpolation (i. e., linear interpolation on  $1/m$ ) is better if  $m$  exceeds 2. If the appropriate number of degrees of freedom is not tabulated, interpolation should be carried out on the difference between  $\chi^2_m$  and the tabular  $\chi^2$ . Suppose, for example, that we wish to find the 1 per cent value of  $\chi^2_m$  for eight degrees of freedom, the smallest expectation being 1. For six degrees of freedom,  $\chi^2_m$  is 17.80 and  $\chi^2$  is 16.81, the difference being 0.99. The corresponding difference for ten degrees of free-

dom is 0.70. Linear interpolation gives 0.84 for eight degrees of freedom. Since the tabular 1 per cent point of  $\chi^2$  is 20.09 for eight degrees of freedom, the 1 per cent point of  $\chi^2_m$  is taken as 20.93. The correct value is 20.95.

With more than one small expectation, as is often the case in testing the goodness of fit to a unimodal frequency distribution, the errors in using the tabular  $\chi^2$  are presumably greater. By an extension of the method above, the probability levels of  $\chi^2_m$ , at the 5 and 1 per cent levels of the tabular  $\chi^2$  were worked out for a goodness of fit test with two expectations of 1.0, the other expectations being assumed large. The corresponding results for one and two small expectations are compared in Table 9.

TABLE 9  
COMPARISON OF THE PROBABILITY LEVELS OF  $\chi^2_m$  WITH ONE AND TWO SMALL EXPECTATIONS ( $m = 1.0$ )

NUMBER OF SMALL EXPECTATIONS	NUMBER OF DEGREES OF FREEDOM IN $\chi^2$						
	3	4	5	6	10	15	25
5 Per Cent Level of the Tabular $\chi^2$							
1.....	.0537	.0553	.0540	.0534	.0527	.0522	.0516
2.....	.0592	.0612	.0585	.0569	.0556	.0545	.0529
1 Per Cent Level of the Tabular $\chi^2$							
1.....	.0144	.0139	.0137	.0136	.0123	.0117	.0111
2.....	.0177	.0178	.0176	.0172	.0147	.0133	.0124

With two small classes, the errors in using the tabular  $\chi^2$  are consistently about twice as great as with one small class. At the 1 per cent level, all the tabular  $\chi^2$  probabilities in Table 9 are in error by more than 20 per cent, though only one value exceeds this limit at the 5 per cent level.

In the above investigation the expectations were treated as if known exactly, whereas in practice they are usually estimated from the sample. Calculation of the exact distribution of  $\chi^2$  in such cases would be illuminating, but would involve laborious computations. At first sight it appears that the errors introduced by a single small expectation are perhaps smaller than those shown in Table 7, since with one unknown parameter a  $\chi^2$  with four degrees of freedom represents six classes, as against five classes where there are no unknown parameters.

I am indebted to Dr. R. L. Anderson for the greater part of the computations in Tables 7 and 8.

## SUMMARY

The  $\chi^2$  correction for continuity is a device for obtaining an approximation to the sum of a number of discrete probabilities by means of a corresponding area under the tabular  $\chi^2$  frequency distribution, and should be used only at the final stage of calculating the probability level attached to a value of  $\chi^2$ . The method of making the correction is illustrated on a common test for linkage in genetical investigations.

Where several values of  $\chi^2$  (each with a single degree of freedom) are added to form a total  $\chi^2$ , uncorrected values must be used.

The effect of a single small expectation in goodness of fit tests is illustrated by working out the exact distribution of  $\chi^2$  for three examples. The discontinuities in the exact distribution of  $\chi^2$  produce only a small part of the discrepancy between the tabular and the exact  $\chi^2$  distributions. By a theoretical approach taking account of the size of the smallest expectation, a more satisfactory approximation is obtained to the exact distribution of  $\chi^2$  and is employed to estimate more generally the sizes of the errors involved in using the tabular  $\chi^2$ . With only a single small expectation, the conventional limit of five for the smallest expectation appears unduly high. At the 5 per cent level, the tabular  $\chi^2$  distribution may be used without undue error with an expectation as low as 0.5, and at the 1 per cent level with an expectation as low as 2. For more exact work, a small table is given of the 5 and 1 per cent levels of  $\chi^2$  as found by the new approximation.

The errors involved where two expectations are small and the effects introduced by the estimation of unknown parameters are briefly discussed.

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# PARASITES OF THE AMERICAN COOT (*FULICA AMERICANA*) IN CENTRAL IOWA<sup>1</sup>

ROBERT L. ROUDABUSH<sup>2</sup>

From the Department of Zoology and Entomology, Iowa State College

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During the 1937 fall migration of waterfowl, a survey of the parasites found in the American coot (*Fulica americana* Gmelin) was undertaken, since few intensive examinations have ever been made on a single species of game bird.

Seventeen birds were collected on two small lakes in central Iowa and examined as soon as possible after being shot. Blood smears were made immediately, and upon reaching the laboratory arthropod, helminth, and protozoan parasites were removed and fixed in the appropriate manner. The survey revealed the presence of four species of protozoa, four species of trematodes, three species of cestodes, two species of nematodes, and eight species of *Mallophaga*.

The primary purpose of this survey is not to give morphological descriptions of the parasites found, but merely to list the parasites and the number and percentage of hosts infected, along with literature references wherever possible. Brief remarks will be necessary to describe those parasites which have not formerly been recorded from the American coot.

## PROTOZOA

### *Eimeria paludosa* (Leger & Hesse)

Examination of the fecal matter of seven coots disclosed the presence of unsporulated oöcysts of a coccidium. Sporulation took place in 4 per cent potassium dichromate in 64 hours and revealed the species to be a member of the genus *Eimeria*. Leger and Hesse (4) described, under the name *Jarrina paludosa*, a species of coccidium from the European coot (*Fulica atra atra*) and the moor-hen (*Gallinula chloropus chloropus*). It is quite apparent that this species and the one found in *Fulica americana* are identical, and since there is no major difference by which it differs from the genus *Eimeria*, the genus *Jarrina* becomes a synonym.

The original description of *Eimeria paludosa* gave the dimensions of the oöcyst as 14  $\mu$  to 15  $\mu$  by 11  $\mu$ , and the spore as 9  $\mu$  by 5  $\mu$ . Size of the form found in survey ranged from 11  $\mu$  to 18  $\mu$  in length and 9  $\mu$  to 13  $\mu$

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<sup>2</sup> Now with Ward's Natural Science Establishment, Inc. Rochester, N. Y. The author wishes here to express his appreciation to Dr. O. Wilford Olsen for checking the identification of the helminths and to Dr. F. H. Wilson for checking the identification of the Mallophaga.

in width, with a mean of 15  $\mu$  by 11  $\mu$ ; the spore ranged from 7 $\mu$  to 12  $\mu$  in length and 4  $\mu$  to 6  $\mu$  in width with a mean of 9  $\mu$  by 5  $\mu$ .

*Haemoproteus* sp.

In one bird several gametocytes of a species of *Haemoproteus* were found, but since the infection was not heavy no definite morphological studies could be made. This is apparently the first report of this genus from the American coot.

*Plasmodium relictum* Grassi & Feletti

A single coot harbored an infection of *Plasmodium relictum*. The infection was very light, but intense examination of blood smears revealed both schizonts and gametocytes. All forms found were typical of the species. Up to the present time there have been no reports of plasmodia in the American coot.

*Trichomonas fulicae* Travis

Travis (9) described a trichomonad from *Fulica americana* and gave an accurate account of the morphology and size of this species. Examination of the caeca of three of the seventeen coots revealed this species of flagellate.

PLATYHELMINTHES

TREMATODA

*Catatropis pacifera* Noble

Upon examining the caeca of five of the seventeen coots, specimens of *Catatropis pacifera* were found. This species was described by Noble (6) and reference to that paper should be made for more detailed information.

*Cotylurus* sp.

An undetermined species of the genus *Cotylurus* was found in the small intestine of two hosts examined. Infection was very light and only five specimens were found in both hosts. Unfortunately, all specimens were flattened at the time of fixation, and so sections could not be cut in order to give it specific identification.

*Cyclocoelium pseudomicrostomum* Harrah

This species of monostome occurred in the air sacs of four out of the seventeen coots examined. It should be pointed out that one bird had as many as 54 parasites while another had only 2. Another species of this genus, *Cyclocoelium microcotyleum* Noble 1933, reported from *Fulica americana*, was not found during this survey.

*Echinostoma revolutum* (Froelich)

In the small intestine of seven of the seventeen birds examined, this species of trematode was found. Of all the flukes found, this species occurred in the greatest number of hosts and in the largest numbers in any host. In recent years this species has been recorded from numerous hosts, and its life history has been delineated.

## CESTODA

*Diorchis americana* Ransom

This species of cestode was found in 15 of the 17 birds examined. Specimens with gravid proglottids averaged 23 mm. in length, had hooks 63  $\mu$  long, and the cirrus was characteristically slender. The American coot is the type host for this species.

*Diorchis acuminata* (Clerc)

Unlike the preceding species this tapeworm occurred in only 10 of 17 birds. The most mature specimens were up to 85 mm. in length. Hooks averaged 40  $\mu$  in length and the extruded cirrus had a bulbous enlargement at its base. Infections with this species were light in comparison with *Diorchis americana*.

*Liga gallinulae* (Van Beneden)

Two immature specimens of *Liga gallinulae* were found in one coot. Although not mature, the principal characters were sufficiently evident to make the identity certain.

## NEMATHELMINTHES

*Amidostomum chevreuxi* Seurat

This species was originally described from the stilt, *Himantopus himantopus*, and although another species has been recorded from *Fulica atra*, the specimens found in *Fulica americana* resemble *Amidostomum chevreuxi* in morphology. Size, however, is one character which differs, the specimens at hand being larger than those described by Seurat (8). Spicules in the specimens collected were 200  $\mu$  long as against 120  $\mu$  in the original description; telamon was 85  $\mu$  long in comparison to 60  $\mu$  for Seurat specimens. In the American coot these parasites were restricted to the area between the corneus lining and the muscular wall of the gizzard.

*Polymorphus* sp.

Numerous specimens of *Acanthocephala*, all apparently belonging to the genus *Polymorphus* were collected from 14 coots.

## ARTHROPODA

## MALLOPHAGA

The following mallophaga were found in this survey (numbers after the species' names indicate the number of individual hosts found parasitized): *Esthiopterus luridum*, 17; *Laemobothrion nigrum*, 10; *Philopterus pertusus*, 17; *Pseudomenopon pacificum*, 16; *Rallicola advena*, 8; *Anatoecus* sp., 1; *Esthiopterus* sp., 1; *Philopterus* sp., 1. The last three species are represented in this collection by single females only. Since the male genitalia are used in classification, these three cannot have specific names applied to them at this time. They probably represent stragglers from other water birds.

The results of this survey reveal the fact that even though individual examinations of a host species bring to light isolated parasitisms, unless intensive studies are carried out the sum total of parasites may never be known. Probably no worker is an authority on all the fields of parasitology essential to compile the necessary information, but it is evident that assistance can be obtained if the parasites are correctly preserved at the time of collection.

TABLE 1  
SUMMARY OF PARASITES COLLECTED FROM SEVENTEEN HOST INDIVIDUALS

SPECIES OF PARASITES	TOTAL NO. OF HOSTS INFECTED	APPROX. % OF HOSTS INFECTED	LOCATION IN HOST
<b>PROTOZOA</b>			
<i>Eimeria paludosa</i> (Leiger & Hesse) 1922.....	7	41	Cæca & colon
<i>Hæmoproteus</i> sp.....	1	6	Blood
<i>Plasmodium relictum</i> Grassi & Feletti 1891.....	1	6	Blood
<i>Trichomonas fulicæ</i> Travis 1936.....	3	18	Cæca
<b>TREMATODES</b>			
<i>Catatropis pacifica</i> Noble 1933.....	5	29	Cæca
<i>Cotylurus</i> sp.....	2	12	Small intestine
<i>Cyclocelium pseudomicrostomum</i> Harrah 1922	4	24	Air sacs
<i>Echinostoma revolutum</i> (Froelich) 1802.....	7	41	Small intestine
<b>CESTODES</b>			
<i>Diorchis acuminata</i> (Clerc) 1903.....	10	59	Small intestine
<i>Diorchis americana</i> Ransom 1909.....	15	88	Small intestine
<i>Liga gallinulae</i> (Van Beneden) 1861.....	1	6	Small intestine
<b>NEMATODES</b>			
<i>Amidostomum chevreuxi</i> Seurat 1918.....	2	12	Wall of gizzard
<i>Polymorphus</i> sp.....	14	82	Small intestine & colon
<b>ARTHROPODS-MALLOPHAGA</b>			
<i>Anatocetus</i> sp.....	1	6	External body surface
<i>Esthiopterus luridum</i> (Nitzsh).....	17	100	External body surface
<i>Esthiopterus</i> sp.....	1	6	External body surface
<i>Læmbobothrium nigrum</i> Burm.....	10	59	External body surface
<i>Philopterus pertusus</i> (Nitzsh).....	17	100	External body surface
<i>Philopterus</i> sp.....	1	6	External body surface
<i>Pseudomenopon pacificum</i> (Kell.).....	16	94	External body surface
<i>Rallicola advena</i> (Kell.).....	8	48	External body surface

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# NATURE OF EIMERIA NIESCHULZI GROWTH-PROMOTING POTENCY OF FEEDING STUFFS. III. PANTOTHENIC ACID

E. R. BECKER AND LAWRENCE SMITH

From the Department of Zoology and Entomology, Iowa State College

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It has recently been demonstrated (Becker and Dilworth<sup>1</sup>) that vitamin B<sub>1</sub> supplement to a ration submarginal in vitamin content exerts a restraining action on the development of the coccidium *Eimeria nieschulzi* in the mucosa of its rat host, if the number of terminal stages (oöcysts) produced during the infection are accepted as the measure of that development. Supplementing with vitamin B<sub>6</sub> instead of B<sub>1</sub>, on the other hand, increases the yields of oöcysts. When the two vitamins are fed together the oöcyst counts decline to levels significantly lower than those for vitamin B<sub>1</sub>. Thus, while it is true that vitamin B<sub>6</sub> may be considered a coccidium-growth stimulant when the ration is deficient in vitamin B<sub>1</sub>, it probably would not play that role in a ration well provided with that vitamin unless, perchance, certain other unknown factors should intervene to nullify the interaction.

In the search for other accessory food factors that might affect the development of the parasite in the host, our attention was drawn to pantothenic acid, the synthetic calcium salt of which is now commercially available. This vitamin is widely distributed in living organisms, both plant and animal (Williams *et al.*<sup>2</sup>), is a growth determinant for rats (Subbarow and Hitchings<sup>3</sup>), its deficiency produces dermatitis in chicks (cf. Williams<sup>4</sup>) and rats (cf. Richardson and Hogan<sup>5</sup>), and it is concerned in nutritional achromotrichia in rats (Gyorgy and Poling <sup>6, 7</sup>). In addition, it is known to be active in the growth of microorganisms; namely, certain bacteria, yeast, and protozoa (cf. Williams and Saunders,<sup>8</sup> Wood *et al.*<sup>9</sup> Elliott <sup>10</sup>). The chemical structure of pantothenic acid is known (Williams<sup>11</sup>), and it has been synthesized (Stiller *et al.*<sup>12</sup>). The synthetic product showed the expected biological activity in chicks and rats.

The plan of the experiment was to test, first, the effect of calcium pantothenate supplement to the control ration, then the effects of vitamins

<sup>1</sup> Jour. Inf. Dis., 63:285, 1941.

<sup>2</sup> Jour. Am. Chem. Soc. 55:2912, 1933.

<sup>3</sup> Jour. Am. Chem. Soc. 61:1615, 1939.

<sup>4</sup> Science, 89:486, 1939.

<sup>5</sup> Proc. Soc. Exper. Biol. and Med., 44:583, 1940.

<sup>6</sup> Jour. Biol. Chem. 132:789, 1940.

<sup>7</sup> Science, 82:202, 1940.

<sup>8</sup> Biochem. Jour., 28:1887, 1934.

<sup>9</sup> Jour. Bact., 33:227, 1937.

<sup>10</sup> Biol. Bull., 68:82, 1935.

<sup>11</sup> Science, 10:246, 1940.

<sup>12</sup> Jour. Am. Chem. Soc., 62:1785, 1940.

<sup>13</sup> Proc. Soc. Exper. Biol. & Med., 46:494, 1941.

$B_1$  and  $B_6$  separately on the supplemented ration, and, finally, the effect of both  $B_1$  and  $B_6$  in the presence and in the absence of calcium pantothenate. The vitamins were administered every other day in amounts as follows: calcium pantothenate 200  $\mu\text{gm}$ .; thiamin chloride, 40  $\mu\text{gm}$ .; pyridoxine hydrochloride, 120  $\mu\text{gm}$ . Administration was in watery solution, directly into the stomach through a rubber catheter attached to a 5 cc. glass syringe. The effects of the vitamins on the infection were measured by numbers of oöcysts eliminated by individual rats during their infections. The table also shows weight gains during the first 16 days on the experimental rations.

Procedure was essentially the same as previously described,<sup>1</sup> except that soybean oil meal was omitted from the ration to which the vitamin supplements were made, leaving the formula as follows, in parts by weight: beet sugar, 74; fine cellulose, 2; medium fine unextracted casein, 15; normal salt mixture (Harris), 4; cod liver oil, 2; lard, 3.

#### EXPERIMENTAL DATA

1. CALCIUM PANTOTHENATE SUPPLEMENT. Eight paired groups of rats, involving in all 64 animals, were employed in the tests to determine the effect of pantothenate supplement to the control ration. Following the previously adopted practice,<sup>1</sup> both the unsupplemented and supplemented series were maintained on their respective rations for 10 days before the date of inoculation, and thereafter until elimination of oöcysts had practically ceased. The size of the groups ranged from 2 to 7 rats. The pertinent data obtained appear in tests 1-8, columns 1 and 2, of Table 1. In every case of the two series, weight gains for the first 16 days on the rations were fairly even, though quite restricted, owing largely to the severe limitation of vitamins  $B_1$  and  $B_6$ . Also, in every test the recipients of pantothenate eliminated more oöcysts than did their controls. With chances equal, the probability of obtaining 8 positives in 8 trials is only about .004. Since the differences in certain cases appeared to be small, the differences between the means of the 8 pairs were tested for significance by the so-called "Student"-Fisher *t*-test, with the result that a probability value less than 0.01 was obtained, indicating that the differences between the two series were highly significant. Pantothenate, then, definitely favored the tendency of the parasite to complete its development in the host when employed as supplement to the control ration.

2. VITAMIN  $B_6$  SUPPLEMENT. Since the coccidium-growth-promoting potency of vitamin  $B_6$  when added to the control ration had already been demonstrated,<sup>1</sup> it was necessary only to test the effect of this material as a supplement to the control ration, which in turn was being supplemented with pantothenate in both test and reference series. The pertinent data appear in tests 10, 11, 12, 14, and 15, columns 2 and 3, of Table 1. The series receiving vitamin  $B_6$  made in general somewhat greater weight gains than the others for the 16-day period. It will be noted that in all 5 tests, involving in all 38 rats, the series receiving vitamin  $B_6$  exhibited a decidedly greater production of oöcysts. The probability of obtaining 5

positives with chances equal is about .031. When the differences between the means of the 5 paired groups were submitted to the "Student"-Fisher test previously mentioned, the probability obtained was considerably less than 0.01. It may thus be concluded that vitamin B<sub>6</sub> in some way or other exerted a markedly stimulating effect upon oöcyst production in the host receiving the control ration plus pantothenic acid.

3. VITAMIN B<sub>1</sub> SUPPLEMENT. Tests 11 and 18, columns 2 and 4, of Table 1, involving 20 rats, showed little or no difference in oöcyst counts, though in one test the B<sub>1</sub> recipients gained considerably more weight than the other lot.

Although vitamin B<sub>1</sub> does exert a depressing effect on oöcyst production when the unsupplemented control ration is fed,<sup>1</sup> it does not seem to do so when pantothenic acid is fed along with that ration.

4. VITAMIN B<sub>1</sub> + VITAMIN B<sub>6</sub> SUPPLEMENT. Since it had been previously shown quite conclusively that vitamins B<sub>1</sub> and B<sub>6</sub>, when fed together<sup>1</sup> or injected intraperitoneally,<sup>13</sup> exerted a marked restraining influence on numbers of oöcysts produced in an infected host on otherwise unsupplemented rations, it seemed highly desirable to ascertain the effect of the two vitamins in the presence of pantothenic acid. The experiment was planned so that in 5 tests (tests 5-9, columns 1, 2, and 5, Table 1) comparisons might be made when the unsupplemented and pantothenate-supplemented rations were used for the control and in 5 other tests (tests 13-17) when either the pantothenate- or B<sub>6</sub>-supplemented rations, or both, were used as controls. The superior host-growth-promoting potency of the pantothenate + B<sub>1</sub> + B<sub>6</sub> combination was clearly demonstrated in most of the tests. The effects of the vitamins on oöcyst production, however, were not so apparent in all cases. The differences between the mean oöcyst counts for the groups in columns 1 and 5 of tests 5-9, Table 1, are not significant. Therefore, it may safely be concluded that in the presence of pantothenate, vitamins B<sub>1</sub> and B<sub>6</sub> did not reduce the oöcyst counts below those for the unsupplemented ration. This conclusion appears further justified by the fact that in 4 of the 5 tests the counts were actually higher for the pantothenate + B<sub>1</sub> + B<sub>6</sub> series.

There are 7 tests (tests 5-8, 13-15) which sought an answer to the question whether vitamins B<sub>1</sub> and B<sub>6</sub> together would reduce the oöcyst counts when both control and test series received pantothenic acid. By inspecting the data for these tests in columns 2 and 5 of Table 1, it can be observed that in 4 of the 7 tests the counts were actually higher in the case of the rats receiving the B<sub>1</sub> and B<sub>6</sub> supplement, while the reverse was true in the other 3 tests. Statistically, the differences are not significant. It appears safe to conclude that in the presence of the pantothenate in control and test rations the other vitamin supplements did not produce an apparent increase in resistance of the host to the completion of the life cycle of the coccidium in its intestine.

5. MISCELLANEOUS TESTS: There appear in Table 1 data for 2 other sets of comparisons. In tests 9 and 13, column 6, appear the data for 2 small groups of rats which received both vitamins B<sub>1</sub> and B<sub>6</sub>, but no

TABLE 1  
MEAN NUMBER OF OCYCSTS ELIMINATED AND MEAN WEIGHT GAINS BY GROUPS OF RATS. (NUMBERS OF RATS IN GROUPS ENCLOSED IN PARENTHESES)

Test No.	KIND OR DATA	RATION DESIGNATION				
		(1) Control	(2) Con.+P.A. +B <sub>1</sub>	(3) Con.+P.A. +B <sub>2</sub>	(4) Con.+P.A. +B <sub>1</sub> +B <sub>2</sub>	(5) Con.+P.A. +B <sub>1</sub> +B <sub>2</sub>
1.....	No. oocysts .....	81 (3)	100 (3)	.....	.....	.....
	16-day wt. gain .....	26 g.	20 g.	.....	.....	.....
2.....	No. oocysts .....	24 (7)	39 (7)	.....	.....	.....
	16-day wt. gain .....	22 g.	21 g.	.....	.....	.....
3.....	No. oocysts .....	58 (3)	77 (3)	.....	.....	.....
	16-day wt. gain .....	29 g.	31 g.	.....	.....	.....
4.....	No. oocysts .....	125 (5)	153 (5)	.....	.....	.....
	16-day wt. gain .....	28 g.	33 g.	.....	.....	.....
5.....	No. oocysts .....	69 (4)	92 (4)	.....	.....	.....
	16-day wt. gain .....	26 g.	26 g.	.....	.....	.....
6.....	No. oocysts .....	90 (4)	96 (4)	.....	.....	.....
	16-day wt. gain .....	35 g.	34 g.	.....	.....	.....
7.....	No. oocysts .....	66 (2)	99 (2)	.....	.....	.....
	16-day wt. gain .....	25 g.	20 g.	.....	.....	.....
8.....	No. oocysts .....	71 (4)	101 (4)	.....	.....	.....
	16-day wt. gain .....	26 g.	26 g.	.....	.....	.....
9.....	No. oocysts .....	34 (1)	.....	.....	.....	.....
	16-day wt. gain .....	30 g.	.....	.....	.....	.....
10.....	No. oocysts .....	.....	.....	43 (5)	87 (5)	9 (2)
	16-day wt. gain .....	.....	.....	31 g.	38 g.	41 g.

TABLE 1—Continued

11.....	No. oöcysts.....	.....	41 (6)	102 (6)	41 (6)	.....
	16-day wt. gain.....	33 g.	33 g.	36 g.	35 g.	.....
12.....	No. oöcysts.....	.....	85 (3)	121 (3)	.....	.....
	16-day wt. gain.....	44 g.	44 g.	44 g.	.....	.....
13.....	No. oöcysts.....	.....	83 (4)	.....	98 (4)	8 (3)
	16-day wt. gain.....	36 g.	.....	.....	49 g.	46 g.
14.....	No. oöcysts.....	.....	22 (3)	81 (3)	66 (3)	.....
	16-day wt. gain.....	25 g.	.....	32 g.	46 g.	.....
15.....	No. oöcysts.....	.....	32 (2)	75 (2)	70 (2)	.....
	16-day wt. gain.....	16 g.	.....	27 g.	40 g.	.....
16.....	No. oöcysts.....	.....	.....	88 (2)	81 (3)	.....
	16-day wt. gain.....	.....	.....	30 g.	33 g.	.....
17.....	No. oöcysts.....	.....	.....	39 (4)	16 (4)	.....
	16-day wt. gain.....	.....	.....	32 g.	42 g.	.....
18.....	No. oöcysts.....	.....	18 (4)	.....	16 (4)	.....
	16-day wt. gain.....	25 g.	.....	.....	39 g.	.....

pantothenate supplement. The oöcyst counts are extremely low as compared with the other rations, as would be expected in the light of previously reported experiments to which reference has previously been made.

Tests 14-17 also make possible a comparison between oöcyst counts for rats on a ration consisting of control + pantothenate + B<sub>6</sub> (column 3) and one consisting of control + pantothenate + B<sub>6</sub> + B<sub>1</sub> (column 5). In all 4 cases weight gains are lower and oöcyst counts higher for the former series. It would require many more experiments to determine positively whether the differences are significant, but it appears certain that vitamins B<sub>1</sub> and B<sub>6</sub> did not reduce the oöcyst counts so materially as they did when pantothenate supplement was not present.

#### DISCUSSION

In continuing the search for food factors that affect the numerical increase of *Eimeria nieschulzi* in its rat host, pantothenic acid has been investigated. This vitamin, like B<sub>6</sub>, promotes rat growth in the proper combination with other vitamins and, when added to our "control" ration, which has a restricted vitamin content, favors coccidium development, though not with the potency exhibited by B<sub>6</sub>. Vitamin B<sub>6</sub>, when added to the pantothenate-supplemented control ration, still further promotes the parasite's development. This apparent independence of the two vitamins is in marked contrast to the parasite-inhibiting effect exhibited by vitamins B<sub>1</sub> (also rat-growth-promoting) and B<sub>6</sub> employed together as the supplement to the same control ration. Future studies may show that it is premature to speak of independent action of B<sub>6</sub> and pantothenate, but at least they should show that they do not operate together to restrict the natural climax of the parasite's cycle.

The oöcyst counts are high, indeed comparable to those for control ration, control ration plus pantothenate, or control ration plus pantothenate and B<sub>6</sub>, when pantothenate is fed in the control ration along with both B<sub>1</sub> and B<sub>6</sub>. The obvious explanation is that pantothenate either masks or interferes with the interaction of B<sub>1</sub> and B<sub>6</sub> that is manifested in reduced oöcyst counts when pantothenate is present in minimal amounts. Quantitative studies on the amount of pantothenate necessary to negate the latter effect would constitute a fascinating study, as would also quantitative studies with B<sub>1</sub> and B<sub>6</sub> irrespective of pantothenate.

The investigation herewith reported centers about the general problem of explaining the potency of certain feeding stuffs in enhancing the reproduction of *Eimeria nieschulzi* in its rat host. Since the number of oöcysts eliminated by a rat during the course of a single infection was adopted as the criterion of the increment of the parasite in the host, the differential effects of the rations are exhibited in terms of forms that represent the culmination of the cycle in the host. Thus, while the study is to be classified as purely in the field of parasite population, the stages of the parasite enumerated were the eliminated products of the infections.

The results obtained are to be regarded strictly as population studies, not as bases for conclusions relative to the effect of diet on clinical coccidiosis.

#### SUMMARY

When a particular ration that was restricted in vitamin B<sub>1</sub>, vitamin B<sub>6</sub>, and pantothenate, and possibly certain other accessory food factors, was employed as "basal," the addition of calcium pantothenate effected moderate increases in the numbers of oöcysts eliminated during *E. nieschulzi* infections.

The additional supplement with vitamin B<sub>6</sub> was evidenced by a still further and more marked increase in oöcyst counts.

The addition of vitamin B<sub>1</sub>, or of both vitamins B<sub>1</sub> and B<sub>6</sub>, to the ration already supplemented with pantothenate did not result in the marked decrease in numbers of oöcysts eliminated so characteristic of the B<sub>1</sub> + B<sub>6</sub> supplement to the ration lacking pantothenate supplement. Thus, pantothenate supplement counteracts the coccidium-inhibiting effect of the vitamin B<sub>1</sub> and B<sub>6</sub> supplement.

#### ACKNOWLEDGEMENT

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# GLUCONIC ACID PRODUCTION BY ACETOBACTER IN THE ABSENCE OF A NEUTRALIZING AGENT

N. PORGES, T. F. CLARK, AND E. A. GASTROCK

From the Agricultural By-Products Laboratory,<sup>1</sup> Ames, Iowa

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Gluconic acid is produced by a relatively simple oxidation of glucose. Such an oxidation can be carried out by chemical or biological means, usually in the presence of a neutralizing agent such as calcium carbonate. However, the ability of certain microorganisms to become adapted to an acid environment offers a means of producing free gluconic acid directly from glucose. A review of the literature indicated that a feasible process for the commercial production of gluconic acid should be able to utilize the oxidizing ability shown by members of the genus *Acetobacter*.

Strains of *Acetobacter* in a medium containing approximately 5 per cent glucose were cultivated in undisturbed solutions and also in vigorously aerated solutions. The results of this preliminary study on acid production in the absence of a neutralizing agent are given in this report. Since the present system of classifying the *Acetobacter* is not perfected, the organisms included in these studies were those reported in previous literature and sub-cultured from original isolations. Unidentified strains obtained from various sources were not included.

## HISTORICAL

Bacterial formation of gluconic acid in glucose media was first reported by Boutroux with *Mycodarma aceti* (13), with *B. pasteurianum* (14), and with an organism isolated from *mycodarma aceti* (15, 16). The calcium salt was found to be identical with that obtained when a glucose solution containing calcium carbonate was subjected to the action of chlorine (34) and bromine (38).

Many additional studies on the bacterial formation of gluconic acid from glucose have appeared. Brown found that this acid was formed by *B. aceti* (17) as well as by his newly isolated *B. xylinum* (18). Another organism produced very little acid directly from glucose according to Zeider (56) unless the acid was neutralized as it was formed. Seifert (45) incubated cultures of *B. pasteurianum* and *B. kützingianum* for 5 weeks and obtained greater yields of acid when calcium carbonate was present. In general, the optimum temperature was between 25° and 30°C. for these bacteria. Variations in temperature influenced acid formation according to Henneberg (27, 28), who used cultures of the three acetic

<sup>1</sup>Established by the Bureau of Agricultural Chemistry and Engineering, U. S. Department of Agriculture, in cooperation with the Iowa State College. The Agricultural By-Products Laboratory was transferred to the Northern Regional Research Laboratory, Peoria, Illinois, July 1, 1941, and work is being continued by the Fermentation Division.

acid bacteria found in *Mycoderma aceti* (*B. aceti*, *B. kützingianum*, and *B. pasteurianum*) and in his newly isolated forms (*B. oxydans*, *B. acetosum*, and *B. acetigenum*). Gluconic acid formation was also observed by Beijerinck (2).

The classical studies of Bertrand (11) showed that his "sorbose bacterium" (later identified as *B. xylinum*) oxidized the aldehyde group of dextrose and other sugars to the corresponding acids and that yields were increased in the presence of calcium carbonate (12). Gluconic acid was also produced by *B. rancens* (3, 35) and by the newly isolated *Acetobacter melanogenum* in the presence of chalk (4). Thermophilic forms of acetic acid bacteria produced little or no acid even in the presence of calcium carbonate according to Waterman (53) and Janke (36, 37).

*Acetobacter suboxydans* was reported by Kluyver and his associates as having the exceptional ability to form gluconic (40) and other acids (41). Visser't Hooft has suggested acid formation from glucose and other substances was a means of classifying the *Acetobacter* (52). Besides *B. xylinoides* and *B. xylinum*, Hermann isolated, from a fermented sweetened tea, a new strain of acetic acid bacteria, *B. gluconicum*, which produced high amounts of gluconic acid (29, 30). Another strain isolated more recently from a similar source also formed gluconic acid (51). Ketogluconic acid is formed in addition to gluconic by *B. gluconicum* (31) and *B. xylinum* (10). Likewise, of four new varieties of acetic bacteria isolated from dried persimmon by Takahashi and Asai (47), three produced other acids in addition to gluconic (48, 49), later shown by Bernhauer and his co-workers to be 2-ketogluconic, 5-ketogluconic (7), and aldehydegluconic (1-guluronic) acids (8). The fourth variety, *B. hoshigaki var. rosea*, showed almost quantitative conversion of glucose to gluconic acid in 10 to 18 days.

It may be observed in the data presented by Hermann and Neuschul (32) that some acetic bacteria do not accumulate calcium gluconate but rather 5-ketogluconate in the presence of calcium carbonate during an incubation period of 90 days. The absence of calcium carbonate tended to prevent the formation of reducing acids (9). On the other hand, almost quantitative conversion of 15 per cent glucose solutions to gluconic acid was reported with *A. suboxydans* in the presence of chalk within 24 hours if the solutions were air-agitated (23).

Gluconic acid has been formed by other bacteria (1, 19, 24) and is recognized as a rather general fungal metabolite. The conditions are given for its formation in flasks (5) and under aeration, pressure, and agitation (25).

The formation of products in addition to gluconic acid has been discussed in the comprehensive survey of the biochemical activities of the acetic acid bacteria prepared by Butlin (20) and others (6, 44).

#### EXPERIMENTAL

Although solutions containing up to 35 per cent glucose have been fermented, calcium carbonate was necessary as a neutralizing agent, and

the addition of boron with the higher concentrations of glucose aided in stabilizing the calcium gluconate (43). However, the production of gluconic acid solutions without the necessity for a neutralizing agent has not been extensively studied, although *B. gluconicum* has been shown to produce the acid continuously by the rapid vinegar method and gave about 80 per cent conversion in 8 to 16 days when a 10 per cent glucose solution was recirculated (33).

The investigation herein reported was made on a 5 per cent glucose medium in an attempt to select an organism, if possible, that would readily convert glucose to gluconic acid in the absence of a neutralizing agent and under conditions of continuous air-agitation. After such selection, additional studies would be necessary to determine the maximum glucose concentration fermentable under varying conditions of pressure, aeration, and agitation.

Cultures maintained for 14 days under undisturbed conditions and cultures aerated for 4 days at the rate of 300 cc. of air per minute per 150 cc. of solution were studied. These incubation periods were selected after experiments had shown that under the conditions of these experiments, most bacterial activity, as measured by glucose utilized and acid accumulated, may be considered as having reached a minimum within the time intervals indicated. In fact, many of the organisms had apparently ceased activity much sooner.

**INOCULUM.** Cultures were maintained on agar slants containing 2 per cent sorbitol (glycerol could be used in place of sorbitol) and 0.5 per cent yeast extract (Difco). After incubation at 30°C. for 7 days, the stock cultures were kept at 5°C. in the refrigerator. Transfers were made every 4 to 6 weeks. Prior to utilization as inocula, the organisms were cultivated for 10 days at 30°C. on agar slants containing 5 per cent glucose, 2 per cent calcium carbonate, and 0.5 per cent yeast extract. After the cultivation period, 10 cc. of sterile water was added to each tube and a bacterial suspension was made by scraping the organisms from the agar surface. The resulting suspension was used as inoculum at the rate of 1 cc. for each 300 cc. of fermentation solution.

**FERMENTATION SOLUTION.** Since a neutralizing agent was not used throughout these fermentations, solutions were prepared to contain approximately 50 grams of glucose and 5 grams of yeast extract per liter. Undisturbed fermentations were made on 300-cc. portions placed in 1-liter Erlenmeyer flasks. Fermentations under aeration were conducted on 150-cc. portions containing 2 drops of octadecyl alcohol added as an anti-foaming agent. The apparatus illustrated in Figure 1 permitted the simultaneous study of the effect of air-agitation on the contents of 32 tubes. The details of a single unit of the aeration assembly are given in Figure 2.

**FERMENTATION.** The flasks and tubes were inoculated in quadruplicate and placed in a constant temperature room at 30°C. for the required fermentation periods. At the end of the fermentation, the pH of the fermented solution in each flask or tube was determined. The solutions, after being made up to their original volumes by the addition of water,

were analyzed for reducing sugars and titratable acidity. Those solutions in each set of quadruplicates which gave approximately the same analysis were combined for detailed analyses. Thymol was added as a preservative. The data obtained on these combined solutions are given in Tables 1 and 2.

**ANALYTICAL PROCEDURE.** The pH was determined by means of a glass electrode. Total acidity was determined using phenolphthalein as the indicator. Standard NaOH (0.1 N) was added so that an excess of about 5 cc. was present over the amount required to give a permanent pink color. The volume was diluted to about 30 cc. and the flask was placed in a water bath until the contents were heated to 60° or 65°C. This warm solution was titrated with HCl (0.1N) and the difference between the alkali and acid readings was recorded as total acidity.

Gluconic acid was determined on a 50-cc. aliquot of the fermented liquor by precipitation as its calcium salt in a 70 per cent alcohol solution similar to the manner described for determining the identification ratio (25). An excess of calcium carbonate was added, and after boiling a few minutes in order to complete the reaction the hot solution was filtered through a Büchner funnel. The residue was washed with hot water. The clear filtrate which contained the salts soluble in hot water was concentrated on a steam bath to a volume of about 20 cc. The concentrate was cooled and sufficient 95 per cent alcohol was added while stirring to obtain a final concentration of 70 per cent alcohol. After cooling the mixture in the refrigerator overnight, the resulting precipitate was filtered on a tared paper in a Büchner funnel and washed five times with cold 70 per cent alcohol. The precipitate was dried at 80°C. for 24 hours and weighed.

The crude calcium salts prepared gave varying reducing values. The tests previously made to obtain the identification ratio indicated practically quantitative recovery of calcium gluconate in the presence of glucose, hence it appeared most likely that this reducing impurity was calcium 5-ketogluconate. It was assumed, therefore, that all reducing material in the crude precipitate was calcium 5-ketogluconate. The difference between this weight and that of the crude precipitate was assumed to be calcium gluconate. Since calcium gluconate contains one molecule of water of hydration, the equivalent amount of gluconic acid can be calculated by applying the factor 0.875.

The amount of acid giving a calcium salt soluble in hot water was determined on 10-cc. aliquots of the fermented solution by boiling with an excess of calcium carbonate, filtering, washing, and determining the calcium in the filtrate by precipitation as oxalate, and titrating with permanganate. Comparing the number of cc. 0.1 N permanganate solution required with that obtained by direct alkali titration of the fermented liquor indicated that in most cases some acids were formed which yielded calcium salts insoluble in hot water.

Calcium determinations made on the crude calcium gluconates showed that all the calcium present in the 70 per cent alcohol solutions was not recovered in these precipitates but that calcium salts soluble in this concentration of alcohol were also formed.

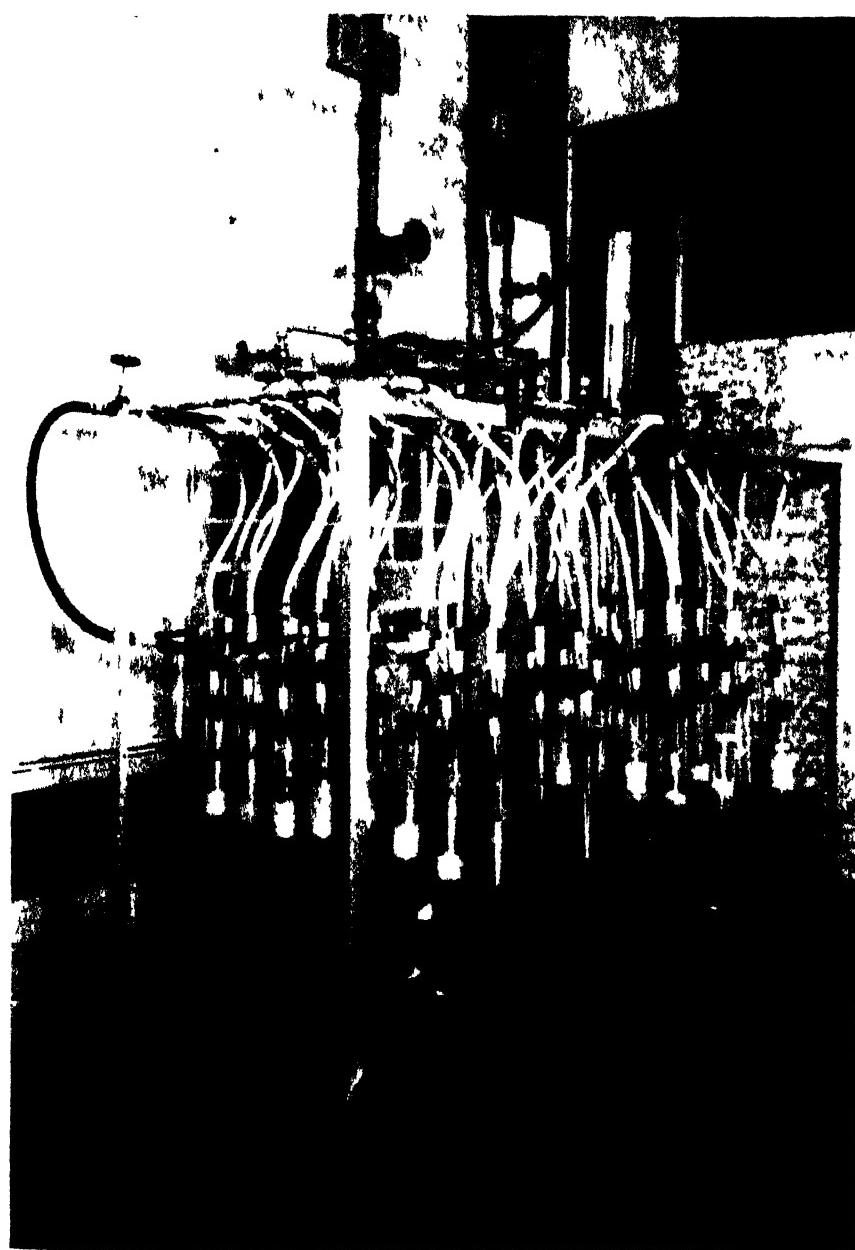


FIG 1. Aeration assembly of 32 units used in fermentation studies.



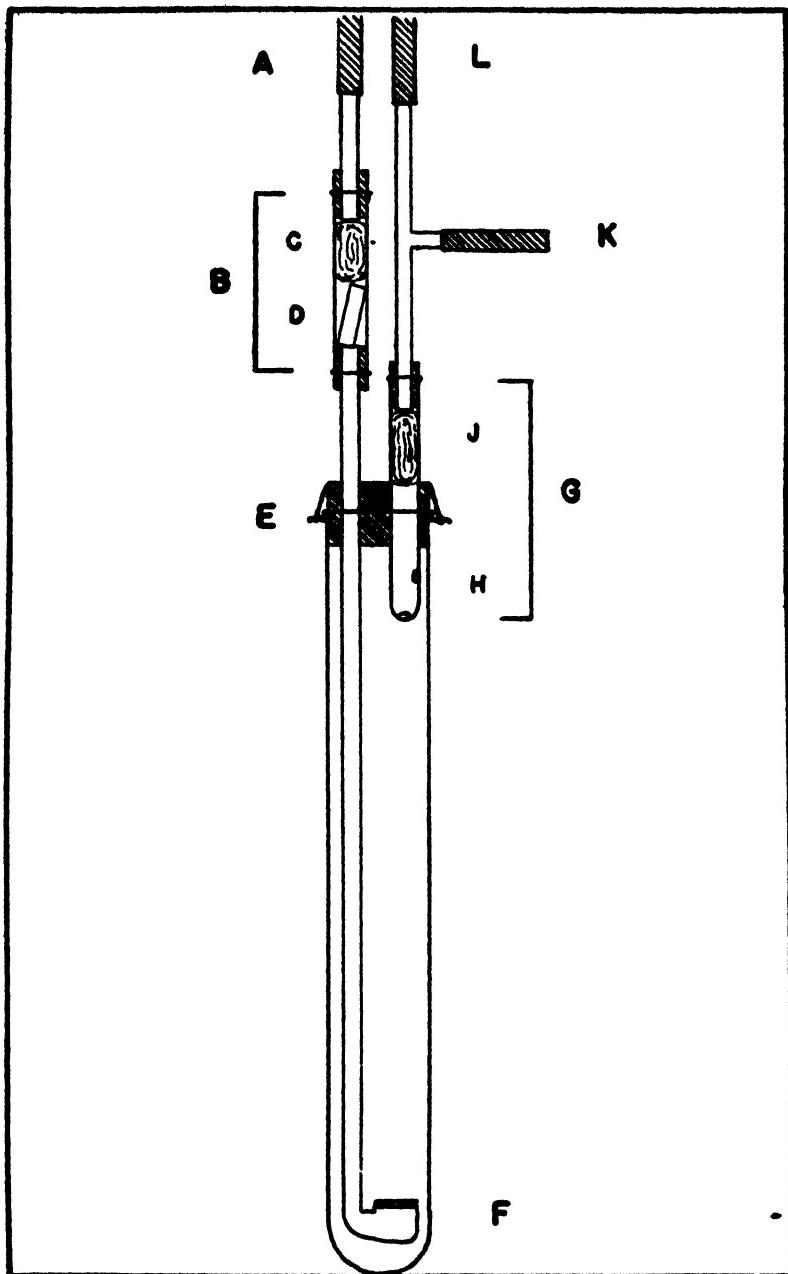


FIG. 2. Details of a single unit in the aeration assembly used in fermentation studies.

- A. Air inlet from compressor.
- B. Tube containing cotton filter C and glass tube D.
- E. Rubber stopper holding glass assembly and held in place by rubber bands.
- F. Sintered glass bubbler.
- G. Air outlet tube with openings at H and cotton at J.
- K. Exit of air into room.
- L. Air passage to flowmeter.

TABLE I  
ACID PRODUCTION OF VARIOUS ACETOBACTER CULTIVATED UNDISTURBED FOR 14 DAYS AT 30°C. DATA PRESENTED ON BASIS OF 100 CC. OF MEDIUM WHICH CONTAINED APPROXIMATELY 5 GRAMS OF GLUCOSE<sup>1</sup>

ORDER OF ACID PRODUCTION	ORGANISM ACETOBACTER	pH	TOTAL TITRATABLE ACID	GLUCONIC ACID <sup>2</sup>	5-KETO- GLUCONIC ACID <sup>3</sup>	VOLATILE ACIDS	Hot Water Insoluble Calcium Salts <sup>4</sup>		ALCOHOL SOLUBLE CALCIUM SALTS <sup>5</sup>	0.1 N
							0.1 N	cc.		
1	suboxydans	2.35	249	201	22	8	22	4	4	4
2	gluconicum	2.56	241	204	4	7	28	5	5	5
3	xylinum	2.50	227	183	6	2	24	14	14	14
4	aceticum	2.52	225	198	.....	6	5	21	21	21
5	acetosum	2.50	221	176	.....	2	25	17	17	17
6	melanogenum	2.20	192	69	16	23	62	45	45	45
7	orleanae	2.56	146	107	21	1	21	.....	.....	.....
8	aceti	2.90	118	113	.....	2	.....	.....	.....	.....
9	ascendens	3.10	87	.....	.....	1	9	7	7	7
10	pasteurianum	3.05	70	63	.....	1	.....	5	5	5
11	rancens	3.12	47	44	.....	0	.....	.....	.....	.....
12	kützingianum	3.30	26	24	.....	0	.....	.....	.....	.....
13	peroxidans	4.74	16	14	.....	1	9	.....	.....	.....

<sup>1</sup>The glucose content per 100 cc. of the sterilized medium was 5.6 grams by the copper method and 5.30 grams by the iodine method.

<sup>2</sup>Obtained by deducting the weight of the calcium 5-ketogluconate from the calcium precipitate and converting the results to gluconic acid.

<sup>3</sup>Assuming that all the copper reducing value of the precipitate is caused by calcium 5-ketogluconate.

<sup>4</sup>Combined calcium insoluble in hot water obtained by subtracting the combined calcium soluble in hot water, calculated as cc. 0.1 N, from the titratable acidity.

<sup>5</sup>Combined calcium soluble in both hot water and in 70-per cent alcohol.

TABLE 1—(Continued)

ORDER OF ACID PRODUCTION	ORGANISM ACETOBACTER	REDUCING VALUE AS GLUCOSE		PORTION OF TITRABLE ACIDITY DUE TO GLUCONIC ACID*	GLUCOSE CONVERTED TO GLUCONIC ACID*	YIELDS
		Copper Method				
		Gram	Gram	Percentage	Gram	Percentage
1.....	suboxydans.....	1.66	1.13	80.6	3.95	64.7
2.....	gluconicum.....	0.95	1.09	84.6	4.01	65.7
3.....	xylonum.....	1.34	1.17	82.5	3.58	58.6
4.....	aceticum.....	1.02	0.91	88.0	3.89	63.8
5.....	acetosum.....	1.48	2.27	79.6	3.44	69.4
6.....	melangenum.....	3.17	8.30	36.0	1.35	56.4
7.....	orleanense.....	3.09	2.88	73.3	2.11	22.1
8.....	aceti.....	3.07	3.30	95.7	2.20	34.4
9.....	ascendens.....	3.89	4.16	81.6	1.40	37.5
10.....	pasteuriannum.....	3.69	3.46	90.0	1.24	36.0
11.....	rancens.....	4.08	4.50	93.6	0.86	25.0
12.....	kützingianum.....	1.93	0.66	92.3	0.47	22.9
13.....	peroxidans.....	4.35	4.91	87.5	0.28	22.1
						20.6
						14.1
						15.3
						7.7
						8.4
						5.0

\*The 5.6 grams glucose present should yield 6.1 grams gluconic acid which is equivalent to 310 cc. 0.1 N titer.

TABLE 2  
ACID PRODUCTION BY VARIOUS ACETOBACTER CULTIVATED IN SOLUTIONS AIR-AGITATED FOR 4 DAYS AT 30°C. DATA PRESENTED ON BASIS OF  
100 CC. OF SOLUTION WHICH CONTAINED APPROXIMATELY 5 GRAMS OF GLUCOSE<sup>1</sup>  
RATE OF AERATION WAS 300 CC. PER 150 CC. SOLUTION PER MINUTE

ORDER <sup>2</sup> OF ACID PRODUCTION	ORGANISM ACETOBACTER	pH	TOTAL TITRATABLE ACID 0.1 N	GLUCONIC ACID <sup>3</sup> 0.1 N	5-KETO- GLUCONIC ACID <sup>3</sup> 0.1 N	VOLATILE ACIDS 0.1 N	HOT WATER INSOLUBLE CALCIUM SALTS <sup>4</sup> 0.1 N	ALCOHOL SOLUBLE CALCIUM SALTS <sup>4</sup> 0.1 N	
								cc.	cc.
1	<i>melanogenum</i> .....	2.06	338	130	55	13	90	63	
2	<i>suboxydans</i> .....	2.30	260	190	25	9	19	26	
3	<i>gluconicum</i> .....	2.35	255	220	9	7	22		
4	<i>orleanense</i> .....	2.28	221	182	19	5	8		
5	<i>acetosum</i> .....	2.47	200	159	12	3			
6	<i>aceticum</i> .....	2.78	145	122	10	2			
7	<i>aceci</i> .....	2.74	116	102	...	...	6	16	
8	<i>xylinum</i> .....	2.88	103	89	8	5			
9	<i>ascendens</i> .....	3.00	62	61	...	1			
10	<i>kuitzingianum</i> .....	3.14	48	46	...	1			
11	<i>pasteurianum</i> .....	3.15	33	25	...	3			
12	<i>rancens</i> .....	3.58	23	19	...	0	5	5	
13	<i>peroxidans</i> .....	5.21	12	8	...				

<sup>1</sup>The glucose content per 100 cc. of the sterilized medium was 5.6 grams by the copper method and 5.30 grams by the iodine method.

<sup>2</sup>Obtained by deducting the weight of the calcium 5-ketogluconate from the calcium precipitate and converting the results to gluconic acid.

<sup>3</sup>Assuming that all the copper reducing value of the precipitate is caused by calcium 5-ketogluconate.

<sup>4</sup>Combined calcium insoluble in hot water obtained by subtracting the combined calcium soluble in hot water, calculated as cc. 0.1 N, from the titratable acidity.

<sup>5</sup>Combined calcium soluble in both hot water and in 70-per cent alcohol.

TABLE 2—(Continued)

Order of Acid Production	Organism Acetobacter	Reducing Value as Glucose		Portion of Titratable Acidity Due to Gluconic Acid	Glucose Converted to Gluconic Acid*	Yield Percentage	Ratio of Weight of Gluconic Acid to 5.6 Grams Glucose
		Copper Method	Iodine Method				
		Gram	Gram				
1	melanogenum	1.19	1.67	38.5	2.56	41.9	45.7
2	suboxydans	1.82	0.72	73.0	3.72	61.0	66.4
3	gluconicum	0.68	0.61	86.3	4.32	70.8	77.2
4	orleanense	1.73	1.20	82.4	3.56	58.4	63.6
5	acetuum	1.50	1.46	79.5	3.12	51.2	55.3
6	aceticum	2.76	2.71	84.0	2.30	37.7	41.1
7	aceti	2.85	4.02	88.0	2.00	32.8	35.7
8	xylinum	4.28	6.69	87.5	1.74	28.5	31.1
9	ascendens	3.78	4.00	98.0	1.19	19.5	21.2
10	kützingianum	4.00	4.19	96.0	1.00	16.4	17.8
11	pasteuriannum	3.76	4.12	76.0	0.49	8.0	8.7
12	rancens	4.80	4.86	82.5	0.37	6.1	6.6
13	peroxidans	3.55	3.99	66.6	0.10	1.6	1.8

\*The 5.6 grams glucose present should yield 6.1 grams gluconic acid which is equivalent to 310 cc. 0.1 N titer.

Volatile acids were determined by extracting a 90-cc. aliquot continuously with ether according to the procedure of McNair (42), who showed that this method avoids the breakdown of other acids. Only small amounts of volatile acids were found in most of the liquors, and qualitative tests showed the presence of acetic acid and only traces of formic acid. An oily residue also was present in the ether extract.

The reducing value of the fermented solution was determined by the Shaffer and Hartmann copper method (46) and also by the Kline and Acree iodine method (39). The residual glucose determined by the copper method should include, in addition to glucose, other reducing substances, such as ketogluconic and guluronic acids. Since the Kline and Acree method determines aldo-groups in the presence of keto-groups, it was believed that the use of the two methods would indicate the amount of keto-acids present. The analyses were made on solutions clarified by means of lead acetate and dipotassium acid phosphate. The reducing values in terms of glucose obtained by these methods showed such variation that the data were useless for determining the amount of keto-acids or of glucose in the liquor. Incidentally, both methods of analysis gave the same results with pure dextrose solutions before and after clarification. The clarified nonfermented medium gave a glucose value of 5.6 grams per 100 cc. by the copper method and 5.3 grams by the iodine method, indicating the presence of copper-reducing substances besides glucose.

Since the amount of glucose utilized by the organisms was not determinable by the methods used, the pertinent data in the tables and figure have been presented for ready comparison on the basis of normality.

#### I. FERMENTATIONS CONDUCTED UNDISTURBED IN FLASKS

The data on fermentations conducted in 1-liter Erlenmeyer flasks containing 300 cc. of solution and left undisturbed for 14 days are in Table 1 and Figure 3. The organisms have been listed in the order of decreasing amounts of total acidity found in solution and show marked differences in their ability to accumulate acid. Although *A. suboxydans* showed the highest amount of total acidity, *A. gluconicum* gave the highest yield of gluconic acid. All the precipitates insoluble in 70 per cent alcohol had some reducing values which were calculated as equivalents of calcium 5-ketogluconate, although other reducing substances were probably present.

The values reported as residual glucose are of doubtful significance, as indicated earlier. In only two cases, *A. suboxydans* and *A. xylinum*, does the difference in reducing values approach that of the calcium 5-ketogluconate values.

It is apparent that marked accumulation of acid occurs only with the organisms that do not oxidize the glucose completely. The calcium complexes varied in color; white products were obtained from cultures of *A. ascendens* and *A. gluconicum*, brown from *A. suboxydans*, while a very dark brown product was recovered from *A. melanogenum*.

## II. FERMENTATIONS CONDUCTED WITH AIR-AGITATION

The use of aeration with this group of organisms is not novel since it has been used efficaciously to yield various products (21, 22, 23, 26, 50, 54, 55).

The inoculated tubes were aerated for 96 hours at the rate of 300 cc.

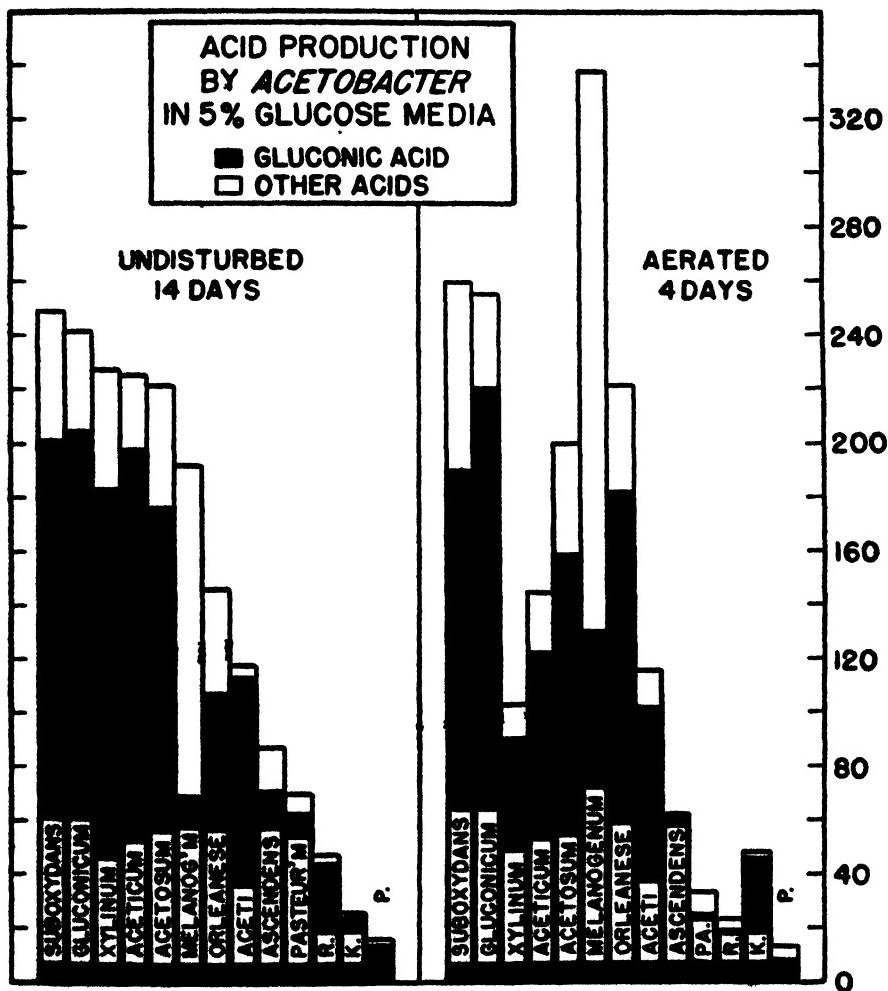


FIG. 3. Total acid, as cc. 0.1 N—per 100 cc. fermented liquor, produced by various *Acetobacter*.

Abbreviations: R.=rancens; K.=kützingianum; P.=peroxidans.

of air per minute per tube of 150 cc. of solution, which was the maximum air flow tried in the preliminary studies. Doubtless, varying this period of aeration would have given data different from those presented in Table 2 and Figure 3. The organisms have been listed in the order of decreasing

amounts of titratable acidity found in the liquor fermented under air-agitation.

It is apparent that aeration had a marked effect on the activity of the organisms as measured by the total acidity produced. *A. melanogenum* shows the greatest increase, and *A. orleanese* also produces more acid under conditions of aeration, while *A. xylinum* and *A. aceticum* show decreased yields. The amounts of volatile acids present show but slight changes from those produced in the undisturbed cultures with the exception of *A. melanogenum* which showed a decrease. In the latter case, a large portion of the calcium was not recovered in the fraction soluble in hot water. The alcohol-insoluble precipitate was deep brown and had a high reducing value. After deducting the calcium keto-gluconate equivalent to the reducing value from the total precipitate, only 38.5 per cent of the titratable acid could be attributed to gluconic acid. Of the remaining high acid-producers, *A. suboxydans* showed the highest content of acids other than gluconic; whereas *A. gluconicum* gave the greatest yield of gluconic and showed increased yields due to aeration, apparently at the expense of the undetermined fraction. In this case, as in the nonaerated tests, the latter organism had relatively small amounts of other acids present.

In this aeration study the calcium precipitates obtained from *A. suboxydans* and *A. melanogenum* were very brown, while the remaining ones were light brown or white.

Aeration influenced the fraction reported as residual glucose. Solutions fermented by *A. melanogenum* and *A. orleanese* gave lower reducing values, while increased reducing values were obtained in liquors containing *A. xylinum* and *A. kützingianum*. The copper and the iodine methods again show marked diversity in reducing value. Aeration decreased those substances that reacted with iodine in the case of *A. melanogenum* and increased those in cultures of *A. xylinum*. The copper-reducing fraction increased with *A. suboxydans*, while it decreased with *A. gluconicum*.

#### DISCUSSION

These studies were made in order to select an organism that would readily convert glucose to gluconic acid in the absence of a neutralizing agent. Five per cent glucose solution was selected in order to permit fairly rapid fermentation, although in the presence of a neutralizing agent (calcium carbonate) concentrations of 15 to 30 per cent of glucose can be fermented. The *Acetobacter* vary in their ability to convert glucose to gluconic acid and, under the conditions of this experiment, form significant amounts of other acids in addition to gluconic, as may be readily observed in Figure 3.

Because this study was limited to the production of gluconic acid, ultimate analyses of the fermented liquors were not made, but traces of formic, small amounts of acetic, and 5-ketogluconic acids were found. In addition, upon adding an excess of calcium carbonate to the fermented liquor, a product, soluble in hot water and insoluble in the 70 per cent

alcohol, was formed. This product had a high degree of hygroscopicity and imparted a brown color to the calcium salts on the filter. *A. suboxydans* formed a relatively large amount of this substance, although it had been reported that this organism formed only gluconic acid when cultivated under intense aeration (23). This product may be the aldehyde-gluconic acid (guluronic) of Bernhauer and Irrgang (8).

The amount of calcium 5-ketogluconate in the precipitated salt was obtained from the reducing value and read directly from a curve, the points of which were previously determined with a pure salt. The presence of other reducing substances doubtlessly affected this value, but in order to estimate the amount of gluconic acid present, the total reducing value as ketogluconate was deducted from the weight of the total calcium precipitate.

Aeration had a varying effect upon acid accumulation. Some cultures gave an increased total acid yield, while others produced lesser amounts. *A. melanogenum* showed the highest amount of titratable acid when the solutions were aerated during incubation, but a large part of this acid was not accounted for in the fractions reported. This undetermined fraction may merit further investigations. Aeration increased the ability of *A. gluconicum* to produce gluconic acid, and under such conditions this organism appeared superior to *A. suboxydans*.

The results have been presented wherever possible as cc. of tenth-normal solutions. For comparison, the 5.6 grams of glucose found in 100 cc. of the original solution should yield, upon complete conversion, 6.1 grams of gluconic acid, which is equivalent to 310 cc. of 0.1 N titer. *A. gluconicum* converted 65.8 per cent of the 5.6 grams of glucose to gluconic acid under undisturbed conditions and increased this conversion to 70.8 per cent under air-agitation. The yields based on the weight of available glucose would therefore be 71.8 and 77.2 per cent.

The final acidity as indicated by the pH of the fermented liquor varied with the organisms and also changed as a result of aeration. The lowest pH, and therefore the highest degree of acidity, was found with those organisms that produced the greatest amounts of acid. In many cases, liquors fermented with air-agitation had lower pH values than their undisturbed counterparts. It is of interest to note that *A. melanogenum* continued its activity at pH 2.06, and that a pH of 2.3 was noninhibitory to at least three species which formed appreciable amounts of gluconic acid. Adjusting the pH of the original solution may offer a means of selecting organisms that produce high amounts of acid and thus withstand high degrees of acidity.

The advantages of using the aeration assembly with selected organisms for a study of this type of fermentation are apparent. Additional studies should be made with use of pressure, in addition to aeration and agitation, in order to ascertain if the fermentation period can be reduced still further and if higher concentrations of glucose can be readily fermented in the absence of a neutralizing agent. The study and control of various factors with a selected organism, such as *A. gluconicum*, should

lead to the establishment of conditions for the production of gluconic acid with the presence of the minimum amount of contaminating acids.

### SUMMARY

Thirteen strains of *Acetobacter* were grown in a 5 per cent glucose solution containing 0.5 per cent yeast extract under two conditions: (a) undisturbed for 14 days; and (b) with vigorous air-agitation for 4 days.

The aerated liquors were generally more acid than their undisturbed counterparts; the lowest pH was 2.06, while three other organisms caused the liquors to reach pH values approximating 2.3.

*A. gluconicum* appeared to be the best organism for the production of gluconic acid in the absence of a neutralizing agent and under conditions of air-agitation.

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# DESCRIPTIONS OF MIMAEAE TRIB. NOV. WITH THREE GENERA AND THREE SPECIES AND TWO NEW SPECIES OF NEIS- SERIA FROM CONJUNCTIVITIS AND VAGINITIS

GEORGE G. DE BORD

From the District of Columbia Department of Health, Washington, D. C.

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The increasing use of cultural methods in place of the microscope in the diagnosis of various pathological conditions in man can be expected to reveal, first, a greater number of organisms which have been described previously but not reported from those sources and, second, many species, both new and old, which resemble morphologically the older reported forms. The diplococcal form is quite common in many pathological conditions. This form may represent either a true diplococcus or the diplococcal form of pleomorphic species. The two types can be separated only by cultural methods so that the various organisms can be placed correctly in a systematic classification of bacteria.

The organisms which form the basis of this report were obtained from cases of conjunctivitis and vaginitis. Those cases included the newborn, children with conjunctivitis and girls of prepuberty age, both normal and those with varying degrees of vaginitis.

## EXPERIMENTAL

The medium used for isolation was a chocolate agar made from Difco proteose peptone No. 3 with 5 per cent citrated human blood. Later rabbit blood was substituted for human blood. Ten per cent sugar solutions in distilled water were sterilized at 15 pounds pressure for 15 minutes. The final dilution in broth was 1 per cent. Aniline oil crystal violet was used in the Gram stain with acetone as the decolorant. All media were incubated before use. A sterile cotton swab was used to obtain the specimen which was streaked immediately over the plate. Each type of colony with some duplications was examined for Gram negative diplococci. The presence of colonies of the genus *Neisseria* was determined by pouring over the surface of the agar an aqueous solution of dimethyl-p-phenylenediamine. Organisms other than *Neisseriae* [Gordon and McLeod (6), Price (12)] give the reaction which makes it essential to examine black colonies microscopically and culturally for identification. •

## DESCRIPTIONS OF SPECIES

### *Neisseria fulva* n. sp.

ETYMOLOGY: *fulvus* (Latin) = tawny, tan, reddish yellow.

Gram negative, nonsporulating, nonmotile diplococci; 0.4-0.7  $\mu$  diameter; morphologically identical to *N. gonorrhoeae*; easily stainable with usual aniline dyes.

**COLONY:** on chocolate agar, raised, round, smooth, margin entire; 1.0-4.0 mm. diameter. No rough variants. Surface of light tan, under surface bright yellow. Colony consistency of soft wax; adheres to substrate. Older colonies show concentric rings.

**DIMETHYL-P-PHENYLENEDIAMINE REACTION:** positive.

**BROTH:** growth granular.

Acid from glucose, fructose, maltose, sucrose.

**SOURCE:** isolated in almost pure culture from eye and vagina of patient with conjunctivitis and vaginitis.

#### *Neisseria gigantea* n. sp.

**ETYMOLOGY:** *giganteus* (Latin) = of or pertaining to giants.

Gram negative, nonsporulating, nonmotile diplococci; 1.0-1.2  $\mu$  diameter; easily stainable with aniline dyes.

**COLONY:** on chocolate agar, raised, round, smooth, margin entire; 1.0-4.0 mm. diameter. Young colonies clear, becoming slightly opalescent. No rough variants. Outer flat rim or margin develops on older colonies. On meat extract agar, opalescent in thicker layers. On both media colonies waxy; colony as a whole can be moved over the substrate with a needle.

**DIMETHYL-P-PHENYLENEDIAMINE REACTION:** positive.

**BROTH:** growth granular, settling to bottom.

Carbohydrates not fermented.

**SOURCE:** normal vagina.

#### SPECIES OTHER THAN NEISSERIAE

In addition to the species of the genus *Neisseria* other species were obtained from the purulent discharge of conjunctivitis and vaginitis which could not be identified by means of the present keys. The diplococcal forms of these species were mistaken for *Neisseriae* in the early part of the investigation, but they were proved later to be pleomorphic rods. The morphology, staining reactions, and the cultural characters of the various species were identical. The fermentative powers varied from absence of fermentation of carbohydrates in some to acid and gas production in several. These species would be placed among several genera if fermentative reactions only were considered, but by so doing the relationship would be so superficial that they would be at variance with species of like fermentative ability.

The morphological and cultural characters of these species with such varying fermentative abilities are the same and may be described as a unit. This unity of characters can serve as a basis of tribal relationship.

The characters in common of the group as a whole are: (1) pleomorphism with the diplococcal form predominating on the solid media and a more nearly even distribution of cocci, rods, and filaments in liquid media; (2) a modified bipolar staining; (3) Gram negativeness with some retention of Gram positiveness; (4) encapsulation; and (5) the physical ap-

pearance of the colonies with various media. Only the fermentative power varies.

When it was believed early in the investigation that these species were *Neisseriae* it was assumed that the occasional rod or filament which was seen was a contaminant. Further platings were carried out in an attempt to purify the culture. However, after repeated platings and examinations of stained smears made from broth cultures, the highly pleomorphic nature of the organisms was fully recognized. The microscopic picture of the freshly isolated organisms is so strikingly diplococcal that a broth culture is useful in showing that the organisms do not belong to the genus *Neisseria*. The cultures which have been grown on artificial media for approximately 12 months have lost the purely diplococcal form on the solid media. The diplococcus/rod ratio has dropped from several hundred/one to one/one or lower. The ratio in broth cultures is less than one/one at the time of primary isolation. No medium has been found which will keep indefinitely the purely diplococcal form on solid media.

Many cells have the appearance of a rod with a modified or extended bipolar staining. The cells appear to be diplococci with adjacent flattened sides but with a very narrow band which is stained very lightly between the two cocci. These cells suggest rods with the polar staining extending somewhat less than one-half the length of the rod. In some of these forms the narrow band is stained so lightly that there is a question whether the cell is a rod or a diplococcus with flattened adjacent surfaces simulating the gonococcus. It is impossible to determine accurately whether some of these forms are truly diplococci or rods with an extended bipolar staining. Many of the forms appear to be diplococci with no suggestion of an unstained narrow band between the two cocci. Either a strong or weak counterstain gives the same picture. This type of staining was observed by D'Herelle (3).

The very lightly stained or the unstained central portion of these organisms is in direct contrast to Escherich's description (4) of *E. coli* in which some of the cells showed a stained central portion with the ends unevenly stained or unstained.

The Gram stain is negative but the staining may be uneven. Some of the cells may retain the blue in whole or in part; however, the retention of the positiveness is not constant. Young and old cultures have shown the retention. Retention has been obtained when either aniline oil crystal violet or Hucker's ammonium oxalate modification is used. Many platings and the fact that individual cells showed a partial retention attested to the purity of the cultures.

Retention of the Gram positiveness has been recorded by Frankel (5), Jaeger (7), von Lingelsheim (10), and Ruata (13).

Encapsulation has been shown consistently over a period of two years on artificial media.

Growth on either chocolate or meat extract agar is luxuriant, and the colonies are white, glistening, smooth, moist, and viscid in varying degrees. With some of the organisms there is not as abundant growth at

TABLE I  
FERMENTATION REACTIONS OF THE PLEOMORPHIC RODS

	Glu.	Mal.	Lac.	Suc.	Mannitol	Dulcitol	Salicin	Dextrin	Motility	Indol	Methyl Red	NO <sub>2</sub>	V-P	Citrate
(a).....	AG	AG	AG	AG	AG	AG	AG	-	+	+	+	+	-	+
(b).....	AG	AG	AG	AG	AG	AG	AG	-	-	+	+	+	-	+
(c).....	AG	AG	AG	-	AG	AG	AG	-	-	+	+	+	-	±
(d).....	AG	AG	AG	-	AG	AG	AG	-	-	+	+	+	-	+
(e).....	AG	AG	AG	-	AG	AG	AG	-	-	-	+	+	-	+
(f).....	A	A	-	-	A	A	VSA	AG	-	-	-	-	-	-
(g).....	A	-	-	-	A	-	VSA	-	-	-	-	-	-	-
(h).....	-	-	-	-	-	-	-	-	-	-	-	-	-	-

± Positive and negative forms were found.

VSA Very slight change toward acidity with phenol red.

primary isolation as later. The growth in meat extract broth is diffuse, usually with a viscid sediment which rises from the bottom in whorls when the tube is shaken.

The differentiation of these organisms is the action upon carbohydrates. The fermentation reactions are shown in Table 1.

If these organisms are combined according to the type of fermentation three separate groups may be made as follows: Group One, those which possess no fermentative power with any of the carbohydrates tested; Group Two, those which ferment with the production of acid only; and Group Three, those which produce acid and gas from the carbohydrates.

#### *Mima* n. gen.

**ETYMOLOGY:** *mimus* (Latin) = mimic, impersonator.

Gram negative pleomorphic rods; may retain partially Gram positiveness; often show modified bipolar staining. Grow well on meat extract media, growth at primary isolation occasionally less abundant. Do not ferment carbohydrates. May or may not give reaction with dimethyl-p-phenylenediamine.

#### *Mima polymorpha* n. sp.

**ETYMOLOGY:** *polymorphos* (Greek) = many forms.

Pleomorphic, nonsporulating, nonmotile, encapsulated, Gram negative rods, 0.5–0.7 x 1.0–3.0  $\mu$ ; diplococci predominate on solid media with high percentage of rods and filaments in liquid media. Many diplococci identical morphologically to *N. gonorrhoeae*. Apparent cocci 0.5–0.7  $\mu$  diameter, filaments variable 0.5–1.5 x 10.0–40.0  $\mu$ . Show modified bipolar staining. May retain partially Gram positiveness.

**COLONY:** on meat extract or chocolate agar, usually luxuriant growth; colonies white, glistening, smooth, moist, viscid.

**DIMETHYL-P-PHENYLENEDIAMINE REACTION:** negative.

**BROTH:** growth diffuse, usually with a viscid sediment.

**CARBOHYDRATES:** glucose, maltose, sucrose, mannitol, dulcitol, salicin, dextrin not attacked.

**M. R.:** negative.

**V. P.:** negative.

**CITRATE:** negative.

**INDOL:** negative.

**NITRATES:** not reduced.

**CATALASE:** positive.

**SOURCE:** normal vagina.

#### *Mima polymorpha* var. *oxidans* n. sp. n. var.

**ETYMOLOGY:** *oxider* (French) = to oxidize.

**CELL MORPHOLOGY:** that of the species.

**CULTURAL CHARACTERS:** those of the species.

**DIMETHYL-P-PHENYLENEDIAMINE REACTION:** positive.

**SOURCE:** normal vagina, vaginitis.

*Herellea* n. gen.

**ETYMOLOGY:** after D'Herelle.

Gram negative, pleomorphic rods, often retaining partially Gram positiveness; many show bipolar staining; motile or nonmotile. Grow well on meat extract media; ferment certain carbohydrates with production of acid only. Nitrates may or may not be reduced.

*Herellea vaginalis* n. sp.

**ETYMOLOGY:** vaginalis (Latin) = vaginal inhabitant.

Pleomorphic Gram negative rods, may retain partially Gram positiveness, nonsporulating, nonmotile, encapsulated. Diplococci predominate on solid media, rods and filaments show high percentage in liquid media. Many diplococci identical morphologically to *N. gonorrhoeae*. Rods often show bipolar staining. Rods  $0.5\text{--}0.7 \times 1.0\text{--}3.0 \mu$ ; filaments  $0.5\text{--}1.5 \times 10.0\text{--}40.0 \mu$ ; apparent cocci  $0.5\text{--}0.7 \mu$  diameter.

**COLONY:** on meat extract and chocolate agar, white, glistening, smooth, moist, viscid, growth usually luxuriant.

**DIMETHYL-P-PHENYLENEDIAMINE REACTION:** negative.

**BROTH:** growth diffuse, usually with viscid sediment.

**CARBOHYDRATES:** acid from glucose, mannitol, dulcitol.

**M. R.:** negative.

**V. P.:** negative.

**NITRATES:** not reduced.

**CITRATE:** positive.

**CATALASE:** positive.

**SOURCE:** normal vagina, conjunctivitis.

*Colloides* n. gen.

**ETYMOLOGY:** kolla (Greek) = glue, ides = like, glue-like, mucoid.

Gram negative, pleomorphic rods. May retain partially Gram positiveness; often show bipolar staining. Grow well on meat extract media; ferment certain carbohydrates with production of acid and gas.

*Colloides anoxydans* n. sp.

**ETYMOLOGY:** an = not, oxider (French) = to oxidize.

Pleomorphic, nonsporulating, nonmotile, Gram negative rods; may retain partially Gram positiveness; encapsulated; often show bipolar staining; diplococci predominate on solid media, high percentage rods and filaments in liquid media. Many diplococci identical morphologically to *N. gonorrhoeae*. Rods  $0.5\text{--}0.7 \times 1.0\text{--}3.0 \mu$ ; filaments  $0.5\text{--}1.5 \times 10.0\text{--}40.0 \mu$ ; apparent cocci  $0.5\text{--}0.7 \mu$  diameter.

**COLONY:** on meat extract and chocolate agar, growth, usually luxuriant, white, glistening, smooth, moist, viscid.

**BROTH:** growth diffuse, usually with a viscid sediment.

**DIMETHYL-P-PHENYLENEDIAMINE REACTION:** negative.

**CARBOHYDRATES:** acid and gas from glucose, maltose, lactose, mannitol, dulcitol. Does not ferment sucrose, salicin, dextrin.

**INDOL:** positive.

**M. R.:** positive.

**V. P.:** negative.

**CITRATE:** positive.

**NITRATES:** reduced.

**CATALASE:** positive.

**SOURCE:** normal, vagina, vaginitis, conjunctivitis.

The outstanding characters of the three genera are their similarity, morphologically and culturally, and the identical appearance of the diplococcal form to *N. gonorrhoeae*. The morphological and cultural description of one genus is applicable to the other two, except the variation in the fermentation of carbohydrates. It appears logical to group the three genera together to form a tribe.

#### Mimeae n. tribe

**ETYMOLOGY:** *mimus* (Latin) = mimic, impersonator.

Gram negative, pleomorphic, motile or nonmotile rods; often show bipolar staining; may retain partially Gram positiveness; many forms show modified bipolar staining. Grow well on meat extract media; may or may not produce acid or acid and gas from carbohydrates. May or may not give dimethyl-p-phenylenediamine reaction.

#### Key to Genera and Species of the Tribe *Mimeae*

a. Do not ferment carbohydrates.

##### Genus I: *Mima*

b. Do not oxidize dimethyl-p-phenylenediamine.

##### Species I: *M. polymorpha*

bb. Oxidizes dimethyl-p-phenylenediamine.

##### Var. I: *M. polymorpha* var. *oxidans*

aa. Ferment certain carbohydrates.

b. Acid but no gas from carbohydrates.

##### Genus II: *Herellea*

##### Species I: *H. vaginicola*

bb. Both acid and gas from carbohydrates.

##### Genus III: *Colloides*

##### Species I: *Col. anoxydans*

A few of the species under (g) in Table 1 are 0.3 to 0.5  $\mu$  in diameter in the diplococcal form. These forms have been placed with the larger organisms for the present.

The author feels that morphological characters should be given the first consideration with respect to tribal description. All species have the same tribal description in morphology, staining reactions, and the ability to show a variation in form on solid and in liquid media. The genera embrace a greater variability in fermentative powers when compared

to other groups as the colon-typoid. It seems rather doubtful whether motility, as shown under (g) in Table 1, would warrant more than sub-specific, at most specific, value.

The fermentation Groups One, Two, and Three and the key have been made rather broad for two reasons. First, a simplification of specific descriptions, as shown for *E. coli* in the 1939 edition of Bergey's *Manual of Determinative Bacteriology*, appears logical and far less confusing and, second, these groups are entirely in a formative stage.

When the organisms of the tribe *Mimeae* are first isolated from inflammatory conditions, the two outstanding features are, first, the predominantly diplococcal form on solid media and the change to a mixture of diplococci, rods, and filaments in broth, and second, the change of form can be switched at will by changes in the media. After cultivation on media for a year or more, the diplococcal form on the solid media has given way somewhat to rod forms, and in broth the filaments do not show the extremely large sizes and variable shapes. The modified bipolar staining does not vary with time. The tendency to retain the Gram positiveness remains but with decreased frequency.

Occasionally organisms are found which resemble the *Mimeae* in pleomorphism and the modified bipolar staining, particularly in catherized specimens of urine. The taxonomic relationship of these organisms to the *Mimeae* has not been a part of this investigation.

The tribe *Mimeae* should be placed under the *Bacteriaceae* for the present. The organisms of this tribe which do not ferment carbohydrates would not answer the familial description of the *Enterobacteriaceae* as defined by Bergey *et al.* (1). It does not appear desirable to place the organisms in separate tribes upon the basis of a single fermentative test or upon the assumption that the source from which the various organisms were isolated would give a positive or negative correlation with a tribe.

#### DISCUSSION

The *Mimeae* resemble the genus *Proteus* in pleomorphism; however, the *Mimeae* are larger and do not have the growth characters of the genus *Proteus*. They resemble the genus *Klebsiella* in encapsulation, but the morphology is so completely different that their relationship would appear to be higher than generic. The extended or modified bipolar staining suggests a relationship to the *Pasteurella*, but dissimilarity of the two types of staining would preclude any more than a distant relationship. The resemblance to the colon group is the lactose fermentation. The high ratio of diplococcal forms, encapsulation, and the staining reactions are dissimilar. Including the citrate utilization test at least two test substances in the fermentative reactions are at variance with the organism of the colon group which it most nearly resembles. With some exceptions the methyl red, Voges-Proskauer, and the citrate utilization tests would suggest the intermediate group of Koser (9). However, the high percentage of diplococcal forms, the pleomorphism, the modified bipolar staining and the Gram retention would preclude that classification.

The close relationship of the various fermentative groups is shown by the following characters: the diplococcal form on the solid media with diplococci, rods, and filaments in broth; the unevenness of the staining reactions; the presence of cells which appear to have an extended or modified bipolar staining; the encapsulation; the pleomorphism; and the appearance of the colonies on solid media. It would appear that this correlation of morphological and biological characters would constitute a combination sufficient to warrant at least tribal designation.

There have been several species described in the literature which may be related to the organisms of the tribe *Mimeae*. *Cocco-bacillus foetidus ozenae* Perez (11), *Coccobacillus conjunctivae* Ruata (13) and *Coccobacillus acridiorum* D'Herelle (3) appear to be nearer the organisms described in this paper with the possibility that *Coccobacillus fusiformis* Karwacki (8) may belong to a related group. The extremely meager descriptions of the earlier species with an indefinite generic use of *Coccobacillus* or variations of it are somewhat confusing in the literature. A careful study of the original articles by Buchanan (2) failed to confirm the use of the name by Leube or Cornil and Babes as reported by De Toni and Trevisan or by Perroncito as reported by Bergey et al. Assuming that *Cocco-Bacillus aracobius perfoetens* Tissier (16) and *Cocco-bacillus foetidus ozenae* proposed as trinomials and the prior use of the generic name to be invalid, *Coccobacillus perfoetans* Tissier (17) might have priority. However, the description of *Coccobacillus perfoetans* is so meager that objections can be raised to making it a valid name. For the same reason objections can be raised against the validity of *Coccobacillus fusiformis*.

The author is inclined to agree with Smith (14) that the generic use of *Coccobacillus* is not valid. The generic use of the word for a particular group and descriptions in the literature of several species which would not be valid would increase the confusion. Further, while the generic use of compound descriptive words of form or growth characters long used in a generic sense would not necessarily invalidate them, it does not seem desirable since any form or growth character may be used to describe many genera. Sternberg (15) recognized this with respect to the word *diplococcus*.

#### SUMMARY

Two new species of the genus *Neisseria*, *N. fulva*, and *N. gigantea* have been described.

A new tribe, *Mimeae*, of highly pleomorphic rods with three genera, *Mima*, *Herellea*, and *Colloides*, has been proposed. Three type species and one variant, *Mima polymorpha*, *Mima polymorpha* var. *oxidans*, *Herellea vaginicola*, and *Colloides anoxydiana*, have been described.

#### ACKNOWLEDGEMENTS

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# ANENT THE ORIGIN OF SWEET CORN<sup>1</sup>

*Zea Mays, L. var. rugosa, Bonaf.*

A. T. ERWIN

*From the Vegetable Crops Subsection, Iowa Agricultural Experiment Station,  
Ames, Iowa*

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Two divergent opinions are held regarding the origin of sweet corn. One is that it is a distinct species and a pre-Columbian food plant of the North American Indian. The other is that sweet corn is a mutation of field corn and a plant of relatively recent origin.

There are four possible sources of information which may throw more or less light upon the history of this plant. These are archeology, Indian legends, genetic studies, and early American literature.

## ARCHEOLOGY

Innumerable specimens of historic maize are to be found in all the leading archeological museums in the United States. With the exception of a single ear, which may readily be accounted for as a mutation, sweet corn is conspicuous by its absence from these collections. This is the reverse of what one would expect, considering the fact that in the pre-Columbian period four-footed domestic animals did not exist on this continent; consequently, maize was grown wholly for human consumption. In brief, the archeological evidence as to sweet corn being a pre-Columbian food plant is negative in character and clearly suggests that sweet corn is a post-Columbian plant.

## INDIAN LEGENDS

Numerous Indian legends have been reported in print which list sweet corn as an Indian food plant. These legends have been carefully and accurately reported by various investigators and constitute an important source of information, provided they are treated as oral legends rather than factual statements. In weighing statements of this character made by the Indians, the fact must be kept in mind that their sense of time is quite vague; and second, that tribal glorification is likely to be a factor.

The writer upon one occasion was given some seed of cowpeas, *Vigna sinensis*, by a "granny" of the Mesquakies, with the solemn assurance that they had been "grown by her people always." Robbins et al., in referring to the Tewa Indians, reports that wheat "was mentioned along with aboriginal food stuffs. It is even introduced into stories to

<sup>1</sup>Journal Paper No. J-770 of the Iowa Agricultural Experiment Station, Ames, Iowa. Project No. 283.

describe pre-Spanish events, the Indians not being sensitive to anachronisms of this kind."<sup>2</sup>

Such experiences are not uncommon with those who have worked among the Indians. In brief, Indian legends may prove valuable as affording a clue, but many of them require confirmation from other sources.

#### GENETIC CONCEPT

Recent genetic studies<sup>3</sup> have demonstrated that the sugary character of the sweet corn endosperm is due to the inability of the plant to complete the formation of normal corn starch or, as has been expressed tersely, sweet corn is field corn in an arrested state of development. Genetic studies have also determined that the absence of this starch forming character is heritable and characterizes the sweet corn group. In support of the mutation theory, a number of cases are on record. Dr. E. W. Lindstrom discovered a single kernel of sweet corn as a mutant in a controlled series of pedigree cultures of dent corn. Four generations of this mutant have been crossed with normal sweet corn and all have proven the original kernel to have been a true mutant of field corn.

Dr. E. M. East concludes that sweet corn varieties do not belong to a single unit but include both dent and flint types which have lost their ability to form starch. East suggests that such mutations may appear in both groups, but they first appeared in the flint type. In this connection it is interesting to note that the only historic<sup>4</sup> ear of sweet corn on record in the United States, so far as we have been able to learn, is of the Golden Bantam type classed by the writer as belonging to the flint corns; while the mutation of Lindstrom was of the dent type. Mutations may also have occurred in the flour type, Prof. A. L. Kroeber of the University of California collected in Peru an interesting pre-Columbian specimen of sweet corn. Prof. G. W. Hendry, of the same institution, regards this ear as a mutation of flour corn, though, to the writer, it appeared to be *Zea amylsaccharata* of Sturtevant. We are informed by Prof. Juleo C. Tello of the Museo de Antropologia, Lima, Peru, that there exists in the Sierra a sweet variety of an undetermined species of maize which the Indians call "Chullpi-Sara." Professor Tello also notes that he has never found the dented type of maize in pre-Columbian tombs.

An interesting but unrecorded sweet corn mutation may have occurred in Iowa shortly after the war between the states. Mr. S. S. Barr,<sup>5</sup> a retired school teacher residing in the vicinity of Davenport, Iowa, was the party in this case. Barr was a keen student of the corn plant and following his experiments with sweet corn reached three conclusions, two of which were far in advance of his time. One was that sweet corn

<sup>2</sup> Robbins, W. W., J. P. Harrington, and B. Freire-Marreco, *Ethnobotany of the Tewa Indians*, U. S. Bur. Am. Ethn., Bul. 55, 1916.

<sup>3</sup> Mangelsdorf, P. C., and R. G. Reeves. *The origin of Indian corn and its relatives*, Tex. Agr. Exp. Sta., Bul. 574, 1939.

<sup>4</sup> Erwin, A. T., *A rare specimen of Zea Mays var. saccharata*, Science, 79: 589, 1934.

<sup>5</sup> The writer is indebted to Mr. G. L. Kurtzweil of Des Moines, Iowa, and Prof. J. C. Cunningham of Iowa State College for access to the unpublished field notes of Mr. Barr.

was a mutation, or what he called a sport of field corn. Second, that its sweetness was due to the arrest of starch formation in the saccharine stage of field corn. His third concept was that sweet corn could be developed from field corn by late planting, by shading the plants during the summer, and harvesting it in the dough stage for at least 7 years. Barr introduced a variety called Silver Sweet, which he claimed was derived from Silvermine field corn by the above method. Pharoah's Dream, a variety of sweet corn, was another one of his productions. In the light of later genetic studies, it seems probable that he was dealing with either sweet corn mutations or contamination which occurred wholly independent of the cultural controls which he set up.

#### EARLY LITERATURE

A number of eastern publications contain statements that sweet corn is an ancient food plant of the North American Indian. These statements appear to rest either directly or indirectly upon the work of Sturtevant.<sup>6</sup> This authority conceived sweet corn to be a distinct species (*Zea saccharata*, Sturt.) and a corn which he states was "secured from the Indians in 1779." This corn he called Susquehannah or Papoon corn. This opinion was based upon a copied article in the *New England Farmer* to the effect that one Richard Bagnall<sup>7</sup> secured Papoon corn from the Iroquois Indians on the occasion of Sullivan's Expedition.

The *New England Farmer* article in turn was quoted from an anonymous article signed "Plymotheus," published in the *Old Colony Memorial*, a local newspaper published at Plymouth, Massachusetts. Plymotheus in turn based his information upon an oral legend of what somebody told somebody 43 years before.

Parker<sup>8</sup> in his interesting volume, "Iroquois' Use of Maize," lists sweet corn as an Indian food plant and refers to the "Journal of Capt. Richard Bagnall." However, upon request of the author for information as to *Bagnall's Journal*, we are referred to Sturtevant, and to date, we have not been able to locate *Bagnall's Journal*, if any exists.<sup>9</sup> Papoon corn appears to be a good example of a statement first reported as an oral legend and finally stated as a fact. A myth may have value as affording a clue to historic data, but it surely cannot be accepted as a historic fact in the absence of corroborative evidence. Do we have such evidence in this case? *Sullivan's Journal* recounts in detail the fact that quantities of field corn or Indian corn, beans, pumpkins, turnips, and potatoes were destroyed on his expedition, but we do not find a line about sweet corn, or sugar corn, as

<sup>6</sup> Sturtevant, E. L., *Sturtevant's notes on edible plants*, ed. by U. P. Hedrick, Albany, J. B. Lyon Company, 1919 (N. Y. State Dept. Agr., Ann. Rpt. 27 (1918-19), v.2, pt. II)..

<sup>7</sup> Also spelled Bagnal.

<sup>8</sup> Parker, A. C., *Iroquois uses of maize and other food plants*, N. Y. State Mus., Bul. 144, 1910.

<sup>9</sup> Subsequent to the submission of this manuscript, we are in receipt of a letter from Edna L. Jacobson, Head, Manuscripts and History Section, New York State Library, as follows: "I find no mention of a journal of Richard Bagnall in the various publications of journals or lists of journals relating to the Sullivan Expedition or in Henriette M. Forbes, *New England diaries 1602-1800*, published in 1928."

it was sometimes called. Another important source of information regarding Sullivan's Expedition is to be found in the diary of the Livermore<sup>10</sup> family. Through the courtesy of Dr. J. R. Livermore of Cornell University, who is a direct descendant of an ancestor who took part in Sullivan's Expedition, the writer was afforded opportunity to examine the evidence presented in this volume. Like Sullivan's diary, it contains numerous references to the destruction of field corn or Indian corn,<sup>11</sup> but sweet corn is not mentioned. The nearest approach is probably the following: "Tuesday, Aug. 17—we destroyed considerable corn, which is in the milk, just fit to roast." However, the fact must be borne in mind that field corn was and still is used by the Indians for this purpose. Field corn is also widely used today for roasting ears in the South. Surely the history of our cultivated plants must rest upon a more secure foundation than a myth, yet it has been so used in a number of recent publications dealing with the history of sweet corn.

Beverly (1722)<sup>12</sup> recites in detail the dent and flint types of maize found in Virginia but does not mention sweet corn. Likewise, William Bartram, son of the noted botanist, John Bartram, traversed the central and southern Atlantic coast region in 1773-75. He records minutely the plants observed and the food plants used by the Indians, including field corn and beans, but sweet corn is not mentioned. The Papago sweet corn mentioned by G. F. Freeman has been referred to as a historic sweet corn of the Papagos. Dr. R. H. Forbes, formerly Director of the Arizona State Agricultural Experiment Station, who accompanied Freeman on a trip to the reservation at the time this corn was found, advises me that the Papago sweet corn was grown adjacent to a field of Indian corn and was regarded by him and Freeman as the result of a cross with imported sweet corn.

The *American Gardeners' Calendar*, published by Barnard McMahon in 1806, was the first comprehensive book on American gardening. He does not list sweet corn, though he resided in Philadelphia, one of the early garden centers of America. His second edition, published in 1818, lists Indian corn, or field corn, for roasting ears. Field corn and beans are repeatedly mentioned, but nothing is said of sweet corn.

Plymouth, Massachusetts, where Plymotheus resided, is not far from Boston, the home of the Massachusetts State Horticultural Society, and also of *Hovey's Magazine*; yet in the Society's premium list for 1838 an award is offered for "Indian corn for boiling," and 10 years later an award was made for "field corn" and also for "sweet corn." Grant Thorburn of New York, the state in which the Indians were supposed to have grown sweet corn, lists in his catalog of 1828 sugar or sweet corn but offered no

<sup>10</sup> Thwing, W. E., *The Livermore family of America*. Boston, W. B. Clarke Company, 1902.

<sup>11</sup> In the early American literature the term Indian corn was applied to field corn. The term is still so used in many New England localities, so we are reliably informed.

<sup>12</sup> Beverly, Robert, *The history of Virginia, in four parts . . . by a native and inhabitant of the place*, 2nd ed., rev. and enl. by author, London, Printed for B. and S. Tooke, 1722.

named varieties of sweet corn. Surely in the half century intervening between this time and Sullivan's Expedition at least a few named sorts would have been introduced, for as Sturtevant states, "many varieties are always an evidence of antiquity of culture." In the *Transactions of the New York State Agricultural Society*, we find the first premium for sweet corn appear in 1843, though the society gave a great deal of attention to maize.

The earliest reference to sweet corn that we have so far located is found in the *Travel Letters* of Timothy Dwight written in 1821, "Maize of the kind called sweet corn is the most delicious vegetable of any known in this country" (New Haven, Conn.).

Tapley<sup>13</sup> cites a reference of Bordley dated 1801 which would antedate the Dwight record. However, the Bordley reference we find is 1823 in the *New England Farmer*, dated June 14 of that year. Apparently Tapley's citation is a typographical error. The fact should be noted that these early references cluster around the '20's of the Nineteenth Century: Dwight's statement, 1821; *New England Farmer*, 1822; Thorburn first listed sugar corn, 1828.

Certain authorities credit pre-Columbian sweet corn to both the United States and Mexico. On three different trips to Mexico, we visited a number of public markets in primitive districts, but did not find sweet corn in any of them. On one of these trips we visited the Maize Exposition held in the District Federal. Here we found excellent displays of maize from numerous states of the Republic, but we were unable to locate a single ear of sweet corn. We would not want to go on record as saying that sweet corn does not exist in Mexico, but it is certainly not an important food plant there. Maize, in some form, is their principal article of diet. In view of this fact, we would hardly expect it to be so conspicuously absent, if some of our cultivated varieties originated there, as has been suggested.

Dr. Richard E. Schultes of the Harvard Botanical Museum, who is temporarily stationed at the Instituto Botanico, Universidad Nacional, Colombia, informs us that sweet corn does not occur in that country, except in a few instances where the seed has been introduced. He also advises that the amylaceous type of field corn predominates. So far as we have been able to learn, no sweet corn mutations from this type are on record.

The early history of sweet corn in the United States is beclouded and involves a considerable element of speculation. In attempting to piece together the scattered fragments, we get this picture of the history of sweet corn. It is a mutation of field corn of post-Columbian origin. Mutations which met with favor among the settlers appeared in the New England states early in the Nineteenth Century.

<sup>13</sup> Tapley, W. T., W. D. Enzie, and G. P. Van Eseltine. Sweet corn. Albany, J. B. Lyon Company, 1834. (N. Y. Agr. Exp. Sta., Geneva. The vegetables of New York, v. 1, pt. III).



# MOSQUITO LIGHT TRAP CATCHES FROM TEN IOWA CITIES, 1940<sup>1</sup>

J. A. ROWE

*From the Entomology and Economic Zoology Section*

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During the summer of 1940 studies were made of the numbers, seasonal occurrence, and species of mosquitoes in ten Iowa cities. Light traps were used to sample the mosquito populations which occurred. These investigations represent one phase of a general survey of Iowa mosquitoes now being undertaken. This paper is a presentation and an analysis of the data taken.

The traps used were of the vertical-suction type, Figure 1, developed by the New Jersey Agricultural Experiment Station as described by Mulkern (7). They were operated by unpaid cooperators at Lansing, Dubuque, Davenport, Muscatine, Burlington, Ames, Des Moines, Council Bluffs, Sioux City, and Ruthven. Each trap-night represents an approximate 12-hour run per trap. Nightly catches were kept in individual paper boxes labeled with the proper date and locality. They were picked up at regular intervals, taken to the laboratory, and the mosquitoes sorted out and placed in smaller boxes for fumigation. Identifications and counts were subsequently made with the aid of a dissecting microscope.

The writer wishes to thank Dr. W. L. Bierring and Dr. Carl Jordan of the Iowa Health Department and Dr. C. J. Drake of Iowa State College for their keen interest and active support of these investigations. For the operation and care of the traps I am indebted to Mr. William Albert, Lansing; Dr. A. J. Entringer, Dubuque; Mr. J. L. Strelow, Davenport; Mr. Clayton Havemann, Muscatine; Dr. E. C. Sage, Burlington; Mr. Harry Stanwood, Des Moines; Mr. W. P. Bailey, Council Bluffs; Dr. W. S. Petty, formerly of Sioux City, and Dr. J. B. Low, formerly of Ruthven.

## A CONSIDERATION OF THE SPECIES TAKEN

Table I shows the total number and species of mosquitoes taken in all the trap catches. Not all the species listed, however, are considered to be important as pests of man or as disease vectors. Most mosquito workers believe *C. apicalis*, *O. signifera*, *T. inornata*, *T. impatiens*, and *U. sapphirina* to be unimportant because of the rarity with which they attack man. Clark (1), Twin (8), and others give only questionable importance to *C. restuans*; their opinions are not shared, however, by King, Bradley, and McNeel (5), Matheson (6), and Dyar (2), who rate *restuans* among the important species. With the exception of the above species, all attack man, and their importance as pests is in proportion to numbers in which they occur.

<sup>1</sup>Journal Paper No. J-948 of the Iowa Agricultural Experiment Station, Ames, Iowa. Project No. 372.

TABLE 1

TOTAL MOSQUITOES TAKEN IN 783 TRAP-NIGHT CATCHES AT TEN IOWA LOCALITIES, 1940

SPECIES	MALES	FEMALES	TOTAL
<i>Aedes campestris</i> D. and K.	8	3	11
<i>dorsalis</i> (Meigen)	64	26	90
<i>dupreei</i> (Coq.)*	0	1	1
<i>nigromaculis</i> (Lud.)	69	88	157
<i>spenceri</i> (Theob.)	0	1	1
<i>sticticus</i> (Meigen)	1	6	7
<i>triseriatus</i> (Say)	14	4	18
<i>trivittatus</i> (Coq.)	132	183	315
<i>vexans</i> (Meigen)	27,162	20,684	57,836
<i>Anopheles punctipennis</i> (Say)	72	208	280
<i>quadrimaculatus</i> Say	4	126	130
<i>walkeri</i> Theob.	3	77	80
<i>Culex apicalis</i> Adams	165	144	309
<i>erraticus</i> D. and K.	7	11	18
<i>pipiens</i> L.	3,653	2,066	5,719
<i>restuans</i> Theob.	805	677	1,482
<i>salinarius</i> Coq.	2,042	3,410	5,452
<i>tarsalis</i> Coq.	5,990	7,210	13,210
<i>Mansonia perturbans</i> (Walk.)	51	48	99
<i>Orthopodomyia signifera</i> (Coq.)*	0	1	1
<i>Psorophora ciliata</i> (Fabr.)	2	19	21
<i>columbiæ</i> (D. and K.)	4	3	7
<i>ferox</i> (Humboldt.)	0	1	1
<i>horrida</i> (D. and K.)	1	2	3
<i>signipennis</i> (Coq.)	5	88	93
<i>Theobaldia impatiens</i> (Walk.)*	0	6	6
<i>inornata</i> (Will.)	176	243	319
<i>Uranotænia sapphirina</i> (O. S.)	147	317	464
Damaged	920	546	1,466
Total	41,497	46,199	87,696

\*New records for Iowa.

The list includes several species known to be vectors of disease-producing organisms. *A. quadrimaculatus* is of major importance as a vector of malaria, and it is believed by some workers that *A. punctipennis* and *A. walkeri* should not be disregarded in this connection. *A. dorsalis*, *A. nigromaculis*, *A. triseriatus*, and *A. vexans* can transmit experimentally the virus of equine encephalomyelitis; and in recent studies by Hammon et al. (3), the viruses of St. Louis encephalitis and western strain equine encephalomyelitis were isolated from *C. tarsalis*.

Table 2 gives the frequencies with which various species were captured while biting and of those taken in trap catches. It is believed that the biting collections indicate to a fair degree the comparative importance of the species as pests in Iowa. Thus, *A. vexans*, *A. trivittatus*, and *A. sticticus* rank high in importance followed by *C. tarsalis*, *A. nigromaculis*, *P. ciliata*, *C. salinarius*, *A. punctipennis*, *P. horrida*, *P. signipennis*, *A. triseriatus*, *P. ferox*, etc. In the trap catches *A. vexans* was again the highest in frequency of catch, but *A. trivittatus* and *A. sticticus* were very low, as were *P. ciliata*, *P. horrida*, and *P. ferox*. The *Culex* species were much



FIG 1 A mosquito trap



TABLE 2

A COMPARISON OF THE FREQUENCIES OF OCCURRENCE IN LIGHT-TRAP CATCHES AND BITING COLLECTIONS OF IOWA MOSQUITOES (FEMALES)

SPECIES	LIGHT-TRAP CATCHES TOTAL 783		BITING COLLECTIONS TOTAL 113	
	Number of Times Taken	% of Total	Number of Times Collected	% of Total
<i>Aedes aurifer</i> .....	none	.....	1	0.09
<i>campestris</i> .....	3	0.4	5	4.4
<i>canadensis</i> .....	none	.....	1	0.09
<i>cinerinus</i> .....	none	.....	2	1.7
<i>dorsalis</i> .....	18	2.2	7	6.2
<i>dupreei</i> .....	1	0.1	none	.....
<i>flavescens</i> .....	none	.....	2	1.7
<i>nigromaculatus</i> .....	57	7.2	15	13.2
<i>spenceri</i> .....	2	0.25	2	1.7
<i>sticticus</i> .....	4	0.5	26	23.0
<i>stimulans</i> .....	none	.....	11	9.7
<i>triseriatus</i> .....	4	0.5	9	7.9
<i>trivittatus</i> .....	71	9.2	45	39.8
<i>vexans</i> .....	635	81.0	83	73.4
<i>Anopheles punctipennis</i> .....	109	13.9	11	9.7
<i>quadrimaculatus</i> .....	75	9.5	2	1.7
<i>walkeri</i> .....	34	4.3	4	3.5
<i>Culex apicalis</i> .....	87	11.1	none	.....
<i>erraticus</i> .....	10	1.2	1	0.09
<i>pipiens</i> .....	235	30.0	5	4.4
<i>restuans</i> .....	270	30.4	6	5.3
<i>salinarius</i> .....	509	65.0	12	10.6
<i>tarsalis</i> .....	515	65.7	15	13.2
<i>Mansonia perturbans</i> .....	27	3.4	none	.....
<i>Orthopodomyia signifera</i> .....	1	0.1	none	.....
<i>Psorophora ciliata</i> .....	16	2.0	14	12.3
<i>columbiæ</i> .....	3	0.3	none	.....
<i>ferox</i> .....	1	0.1	8	7.0
<i>horrida</i> .....	2	0.25	10	8.8
<i>signipennis</i> .....	50	6.3	10	8.8
<i>Theobaldia impatiens</i> .....	4	0.5	none	.....
<i>ornata</i> .....	91	11.6	none	.....
<i>Uranotaenia sapphirina</i> .....	122	15.5	none	.....

higher in frequency in the trap catches than in the biting collections. These trap catches give a fairly accurate picture of the populations of important species which occurred in the trap vicinities; they should not, however, be expected to give a picture applicable to the whole state. If traps had been operated in all sections of the state and in all types of habitats, the numbers of some important species would probably have been much higher. It is believed that *A. trivittatus* and *A. sticticus* are much more common and occur in larger numbers than is indicated in these trap catches.

#### TRAP CATCHES IN RELATION TO HUMAN COMFORT

Probably the most important activity of mosquito control work is that directed toward the elimination of larval breeding areas. This task is often complicated because such areas are sometimes well hidden and in out-

of-the-way places, and because certain mosquitoes fly considerable distances from their breeding grounds. However, with a thorough knowledge of the breeding and flight habits of the species in a given area, fairly accurate judgments can be made regarding their sources. Prior to about 1927, knowledge of the compositions and densities of mosquito faunas were largely based on adult catches made by human collectors. It soon became evident that data taken in this manner were inaccurate due to differences in the attractiveness to mosquitoes, skill in catching, and diligence among the collectors. To overcome this inaccuracy the New Jersey Agricultural Experiment Station set out to devise a mechanical trap that would catch mosquitoes in a uniform manner under all conditions. At least six types of traps were made over a period of eight years, each one an improvement over the previous one. In 1933 the Model 50 was completed and has been used, with perhaps minor changes, by many mosquito control associations since that date.

A review of the development of mosquito traps and data regarding the interpretation of trap catches were published by Headlee (4) and Mulhern (7). It was found that most important, in the use of traps as sampling devices, was the proper evaluation of trap catches with regard to human comfort. Headlee (4) presented considerable data to show that, under New Jersey conditions, the mechanical trap caught twice as many mosquitoes for the same period as was caught by an expert collector. Also, that a fairly accurate picture of their actual abundance could be derived from the trap catches. A catch of 24 females per night was equivalent to the same density present when 1 female could be caught each 15-minute period by an expert. The 24 female trap catch was considered and has been used for several years as the point of minimum density at which the average individual becomes conscious of mosquito annoyance. It is obvious that such an annoyance figure might be a variable one according to the locality in which traps were operated. VanDerwerker (9) published data to show that this figure may be as low as 8 females in metropolitan areas having extensive control work and as high as 40 in areas where no control work had been done. He also pointed out that a catch of 5 or even 3 females of certain species might indicate annoyance in some situations.

Studies are in progress to determine a trap-annoyance figure for Iowa conditions. A limited amount of data at hand suggests that during periods of increased mosquito abundance, initial complaints by the public are produced when trap catches are somewhat higher than 24. However, when general awareness to annoyance is aroused, complaints are common when catches are less than 24. Until sufficient data have been taken to permit the establishment of a definite trap-annoyance figure for Iowa, that of 24 females per night has been adopted.

#### FACTORS INFLUENCING TRAP CATCHES

Mosquito workers in general realize that several factors greatly influence the activity of the adults, and recognize that a close relationship exists between these factors and the nightly fluctuations of trap catches.

Headlee (4) showed that adult activity, measured by light trap catches, was greater in the evening hours before about 9 p. m. and again in the early morning hours at dawn. It is obvious that the inhibition of adult activity during these periods would produce lower catches in the traps. The general effect of certain factors upon the adult activity during the dusk and dawn periods is known, but the data on specific factors is somewhat limited. Headlee's studies indicated that mosquitoes fly with light winds but cease to fly when the velocity rises above 10 miles per hour; also, that high atmospheric moisture favors flight but that rain causes a reduction in adult activity. He stated further that temperatures lower than 60°F. inhibited mosquito flight. VanDerwerker (10) pointed out that when mosquito abundance is to be measured by trap catches, considerable care should be exercised in the choice of trap locations. He emphasized the importance of placing the traps in protected spots preferably on the outskirts of a town, and in the proper position with regard to wind direction, to permit the interception of mosquitoes flying into a populated area. He also stressed temperature as an important factor in trap catches and presented data to support a theory that the annoyance figure (for Union County, New Jersey) appeared to vary 2 mosquitoes per Fahrenheit degree of temperature change, increasing with high minimum and decreasing with lower minimum temperatures.

It is evident that the correct interpretation and evaluation of the trap catch, as an index to mosquito density can be made only when all the factors existing in the trap area and influencing the catch are known. During these studies an effort was made to place the traps in protected places as close to residential areas as possible. Descriptions of these areas are included under the trap discussions. Funds were not available, however, to permit detailed studies of mosquito breeding or the recording of climatological data at the site of the traps. Most of the trap areas were visited twice each month for a general check on the larval breeding occurring within an area of about 5 miles of the traps. No inspections, however, were made of the islands lying in the Mississippi River, and undoubtedly mosquitoes produced on these entered the traps operated in the eastern cities. Climatological data used in the discussions of seasonal occurrence included herein were taken from the U. S. Weather Reports for each trap locality except as otherwise stated. In view of the limited amount of data at hand on the factors which influenced these trap catches, no attempt has been made to definitely account for the nightly fluctuations of the catches. The seasonal records are intended to be of a general informative nature rather than a critical analysis of the factors which produced the catch fluctuations.

#### TRAP RECORDS FROM LANSING, 1940

Lansing, Iowa, is a small town of about 1,300 people in the northeastern part of the state. It is situated on the west bank of the Mississippi River in the mouth of a narrow valley which extends westward. The country northwest, west, and south of the town is very hilly and wooded; that to

TABLE 3  
TRAP RECORDS FOR LANSING, IOWA 1940 (FEMALES)

Species which rarely bite humans.

<sup>a</sup>Not including species marked with asterisk

the north is largely marshes, lakes, and bayous in the river bottoms; and to the east and southeast is the river with numerous wooded islands. The trap was placed under a row of tall trees near the shore of the river a short distance from the boat houses of the Iowa Conservation Commission. Little or no shrubbery or tall plant growth was near the trap, but the trees and buildings gave some protection from wind. No survey for larvae breeding was made, but from their general appearances the areas northeast and southeast of the trap were believed to be the ones most likely to produce mosquitoes.

The mosquitoes caught during 77 trap-nights are shown on Table 3.

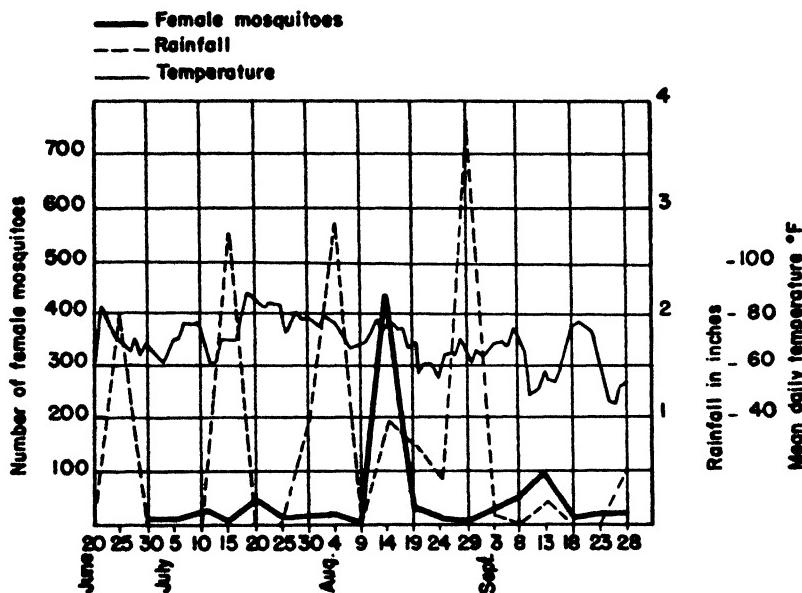


FIG. 2. Trap records, precipitation, and temperatures for Lansing, Iowa, 1940. Mosquito catches are averaged for 5-day periods, precipitation are totals for the same periods, and temperatures are daily means.

*A. vexans* was by far the most common species, *C. salinarius* was second, and *A. quadrimaculatus*, third. The average nightly catch was more than 3 times as high in August as in July or September; that for the entire season was 41. Of the total number taken 87 per cent were *Aedes*, 11 per cent *Culex*, and 2 per cent *Anopheles*. Species which habitually bite man made up 99 per cent of the total catch and on 20 out of 77 nights, or 24 per cent, 24 or more females were taken.

The seasonal record is shown on Figure 2. Two periods of increased mosquito abundance were recorded, August 9 to 14 and September 8 to 13. Both of these were due to *A. vexans*. The first peak followed a precipitation period from July 26 to August 4 and the second followed heavy rains on August 25 to 28. Conditions were favorable for flight during the August peak when fairly high temperatures prevailed (Decorah records) and

TABLE 4  
TRAP RECORDS FOR DUBUQUE, IOWA. 1940 (FEMALES)

AEDES		ANOPHELES		CULEX		OTHERS		TOTAL		AV. NIGHTLY CATCH		NO. NIGHTS CQ.		% ANOPHELES		% CULEX		% WHICH HABITUALLY					
May.....	3	1,109	1	1	1	14	156	17	340	411	4	21	34	2,110	111	14	53	0.1	44	98			
June.....	19	5	290	3	16	1	25	1	329	43	517	464	2	111	197	2,004	111	16	15	1	69	93	
July.....	18	5	22	3	3	1	1	1	58	2	29	15	1	23	24	178	89	2	12	2	60	87	
Aug.....	2	7	73	2	6	1	1	1	55	5	20	4	1	1	1	168	24	2	43	5	44	99	
Sept.....	7	7	73	2	6	1	1	1	55	5	20	4	1	1	1	168	24	2	43	5	44	99	
Total.....	49	1	5	1,494	6	26	2	40	2	598	67	906	894	7	156	256	4,460	91	34	34	1	56	95
Total males.....	1	1	365	4	4	43	5	700	32	351	335	335	335	88	377	2,301	47	16	16	0.2	64	64	

\*Species which rarely bite humans.

\*Not including species marked with asterisk.

the rains were scattered. During the September peak, however, the temperatures were very low; the minimums being below 40°F. from September 10 to 13. The prevailing direction of wind for June, July, and September was southwest, or in an unfavorable direction with regards to the probable breeding area. In August it was from the southeast or in a favorable direction.

#### TRAP RECORDS FROM DUBUQUE, 1940

The trap was located in the eastern part of the city (near the junction of 4th and Pine Streets) in a commercial area situated on the bottom lands bordering the Mississippi River. The site of the trap was a vacant lot choked with tall weeds and shrubs; several trees were also present a distance of about 100 feet away. The main business district was about a quarter mile west of the trap, the eastern residential district, 1 to 3 miles north, and the western residential district, on wooded bluffs overlooking the river, was 1 to 3 miles west. Numerous ponds of various sizes were scattered throughout the area in the vicinity of the trap, most of which supported dense growths of aquatic floating plants and were bordered by cattails, willows, and other shrubs. Some of the ponds were used for dumping areas.

Table 4 shows the composition and numbers of females taken on 49 trap-nights. Motor trouble prevented the successful operation of the trap during August, and it was run only 2 nights. An average nightly catch of 111 was about the same for June and July; for the entire period it was 91. On 34 nights, or 64 per cent of the total, 24 or more females were taken. More *A. vexans* were taken than any other species, but only 34 per cent of the total were *Aedes*, while 56 per cent were *Culex*. It appears that much of the annoyance at Dubuque is due to *Culex* species breeding in the ponds in the eastern part of the city. There is no doubt that control measures applied to these ponds would relieve much of the annoyance. Not only could the *Culex* breeding be stopped but also the considerable *Aedes* breeding which occurs around their edges could be reduced.

Figure 3 shows the seasonal record of female catches, together with precipitation and mean daily temperatures. Four periods of increased mosquito activity are evident. The first period, June 5 to 10, was largely due to an *A. vexans* flight which occurred on June 6 and 7, following a period of precipitation in the latter part of May (not shown). This was the only period during which the bulk of the catches were *Aedes*. The later peaks of activity, June 25 to 30, July 5 to 10, and July 30 to August 4, were caused by the increased breeding of *Culex*. It is interesting to note that periods of increased mosquito activity were generally those of higher mean temperatures. Considerable rain fell in the periods of June 20 to 25 and July 25 to 30, which probably interfered with adult mosquito activity.

#### TRAP RECORDS FROM DAVENPORT, 1940

Davenport is one of the largest commercial and industrial cities of Iowa with a population of about 66,000. It is located on the northern

TABLE 5  
TRAP RECORDS FOR DAVENPORT, IOWA, 1940 (FEMALES)

	No. NIGHTS TRAP	OPERATED	AEDES	ANOPHELES	CULEX		OTHERS	TOTAL	AV. NIGHTLY CATCH	No. NIGHTS COL.	EXCEDED 24†	% ANOPHELES	% CULEX	% WHICH HUMANS	BITE HUMANS		
					VEXANS	STRICTUS											
May.....	3	27	2	1	1,488	9											
June.....	27	27	6	1	3,131	4	32	36	262	125	1	4	20	1,976	73	19	
July.....	27	27	6	1	3,331	5	1	19	3	312	51	495	1,050	11	1	38	
Aug.....	27	27	6	1	205	8	1	1	1	496	7	201	55	3	1	16	
Sept.....	6	6	1	1	121	7	53	8	1	121	7	53	8	1	1	2	
Total.....	90	8	1	3	5,164	20	7	2	19	4	961	107	1,016	1,239	15	1	60
Total males.....											1,078	61	626	2,443	3	1	16

\*Species which rarely bite humans.

†Not including species marked with asterisk.

banks of the Mississippi River, about midway between the north and south borders of the state. The city is situated on the bottom land adjacent to the river and on the bluffs to the north; the western section is partly industrial and partly residential. The trap was located in this area at the city sewage disposal plant; its site was that of a small well-kept garden of flowers and shrubs. South of the trap, a distance of about 200 feet, was the willow-lined flood plain of the river; southwest about 1.5 miles, was a large, flat, wooded island which has been made into a city park. Extending west of the trap was a low, vacant area and to the north, an industrial

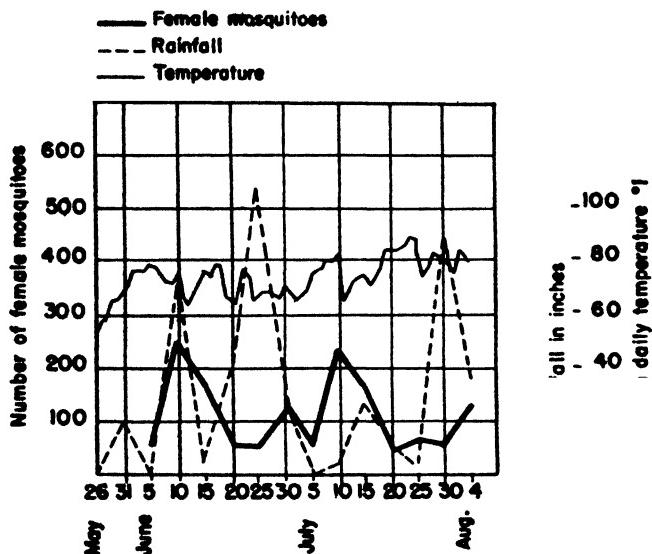


FIG. 3. Trap records, precipitation, and temperatures for Dubuque, Iowa, 1940. Mosquito catches are averaged for 5-day periods, precipitation are totals for the same periods, and temperatures are daily means.

and residential district. Considerable *Culex* and *Anopheles* breeding occurred in a large pond a short distance north of the trap, and in the bayous along the river. Concentrated *Aedes* breeding was found, during certain periods, on the island and in the vacant district west of the trap.

Table 5 shows the composition and numbers of females taken on 90 nights of trap operation. The bulk of the total catch was *A. vexans*; *C. tarsalis* and *C. salinarius* also were taken in considerable numbers. Of the total catch 59 per cent were *Aedes*, 39 per cent *Culex*, and *Anopheles* comprised only 0.3 per cent. Probably the most interesting feature of the catch record was that on 73 per cent of total trap-nights, 24 or more females were taken; all of these were species which habitually attack man.

The seasonal occurrence of mosquitoes recorded by the Davenport trap for 90 nights of operation between May 31 and September 8 is shown in Figure 4. During this time two periods of increased abundance occurred. The first, which began about June 22 and reached a peak on July 8, was

largely due to *A. vexans* produced by two precipitation periods, which occurred between June 11 and June 23. The 15-day period preceding this peak was favorable to mosquito flight. The second period began July 20 with a peak on July 27. It was also caused by *A. vexans* following a precipitation of about 1 inch on July 11. During the last 2 days (July 26 to 27) of this period, however, considerable rain fell which probably interfered with the adult activity. The periods of extensive rainfall during August undoubtedly produced another peak of abundance in September, as was the case in some of the other traps (Ames and Des Moines). This is sug-

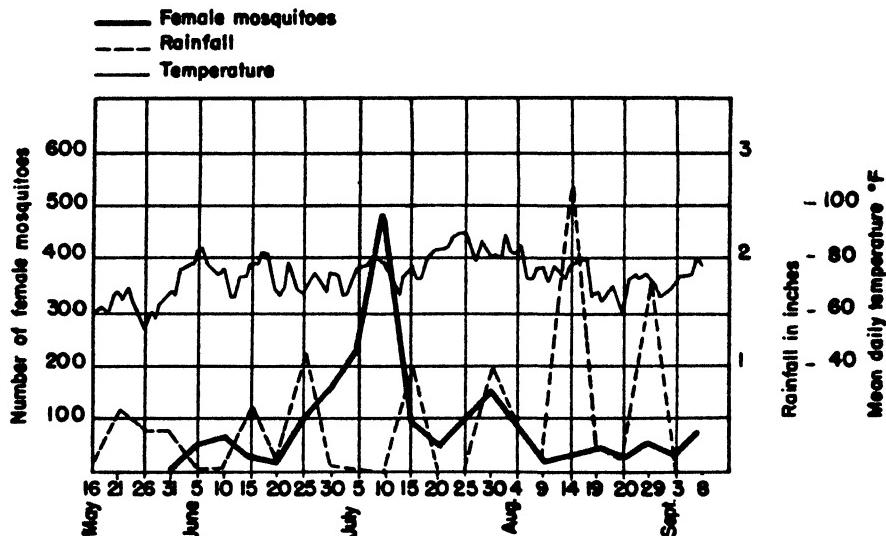


FIG. 4. Trap records, precipitation, and temperatures for Davenport, Iowa, 1940. Mosquito catches are averaged for 5-day periods, precipitation are totals for the same periods, and temperatures are daily means.

gested in the rise between September 3 and 8. Southwest winds during July, August, and September favored the flight of *Aedes* from their breeding areas toward the city.

#### TRAP RECORDS FROM MUSCATINE, 1940

Muscatine, Iowa, has a population of 18,000 and is located on the Mississippi River about 30 miles west of Davenport. The main residential and business districts are situated on the sides of the bluffs overlooking the river. Near the southwest part of the city the river bends southward away from the bluffs producing a rather broad flat valley; the southwest residential district extends into this valley for some distance. The trap was located in this residential district at the home of Clayton Havemann, 1020 Nebraska Street. It was situated in a garden containing the usual flowers, shrubs, trees, etc. The known mosquito breeding areas which were expected to influence the catches were located west and southwest of the trap within a radius of 3 miles. These were mainly intermittent marshy

areas bordering the Muscatine Slough, and occupying low swails which marked previous stream channels. In the river about a half mile east of the trap was a sizable island (Towhead Island) which was not examined for mosquito breeding. The area south of the trap was largely agricultural and industrial and contained no important breeding areas.

Table 6 is a summary of the trap catches for 86 trap-nights. *A. vexans* was the dominant species, and of the total trap catch, 66 per cent were *Aedes*, 30 per cent *Culex*, and only 2 per cent *Anopheles*. The average

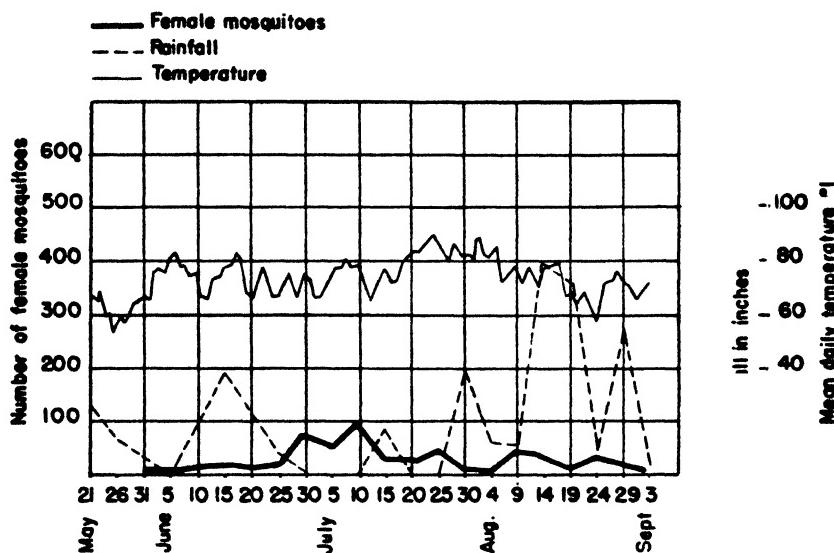


FIG. 5. Trap records, precipitation, and temperature for Muscatine, Iowa, 1940. Mosquito catches are averaged for 5-day periods, precipitation are totals for the same periods, and temperatures are daily means.

nightly catch was only 30 females, and on 50 per cent of the nights 24 or more were taken. *M. perturbans* were taken in larger numbers in this trap than in any other. The record of *O. signifera* is new and the only one from the state.

The trap catches for the 86 days of operation, between May 27 and August 30, were rather small. Figure 5 shows that no periods of unusual mosquito abundance were recorded. The low peaks of June 30 and July 10 were largely due to *A. vexans*, which were produced by precipitation on June 11 to 12, and 18. The lower average catch for the 5 days ending July 5 is partially explained by the lower temperatures of July 2, 8, and 4, the minimums of which were 54°, 56°, and 59° F., respectively. On August 31 the motor of this trap burned out and could not be replaced. It is known from field studies, however, that perhaps the largest peak of abundance occurred in September, following the heavy rains of August. The prevailing winds in June, July, and September were from the south and in August from the east. These directions were unfavorable for flights into the city from the western breeding areas.

TABLE 6  
TRAP RECORDS FOR MUSCATINE, IOWA, 1940 (FEMALES)

	No. NIGHTS TRAP	OPERATED	No. NIGHTS CATCH	EXCEEDED 24†	No. NICHOLS COL.	% ANOPHELES	% Aedes	AV. NIGHTLY CATCH	No. NIGHTS CATCH	% Culex	% WHICH HUMANS	% WHICH HUMANS
						AEDES	ANOPHELES	CULEX	OTHERS	TOTAL	Damaged	
May.....	5	....	8	....	2	....	1	1	....	1	14	3
June.....	27	....	469	10	7	2	5	26	63	55	23	680
July.....	29	1	826	7	8	1	4	157	84	116	5	1
Aug.....	25	2	13	373	4	11	....	1	3	163	21	45
Total.....	86	3	13	1,676	21	26	5	10	3	347	169	217
Total males.....	1	5	1,340	36	3	1	15	1	1,597	286	454	42
											14	3
											6	3,852
											45	....
											35	1
											62	....

\*Species which rarely bite humans.

†Not including species marked with asterisk.

TABLE 7  
TRAP RECORDS FOR BURLINGTON, IOWA, 1940 (FEMALES)

**\*Species which rarely bite humans.**

Not including species marked with asterisk.

TABLE 8  
TRAP RECORDS FOR AMES, IOWA, 1940 (FEMALES)

AEDES	ANOPHELES	CULEX	OTHERS	TOTAL		% ANOPHELES	% CULEX	% WHICH HUMANS BITE HABITUALLY
				No. NIGHTS	OPERATED			
May.....	11							
June.....	26			157	1	8	0.7	0
July.....	22			73	3	1	1	96
Aug.....	30			494	16	241	9	97
Sept.....	25			3,808	31	191	9	99
Oct.....	10			125	7	11	0	43
Total.....	124			4,657	57	43	2	99
Total males.....	1	19	2	77	4,829	8	12	32
						54	27	99
						246	175	88
						1	1	1
						36	37	38
						5	17	80
						61	24	1
						6,674	54	74
						...	...	...

\*Species which rarely bite humans.

†Not including species marked with asterisk.

## TRAP RECORDS FROM BURLINGTON, 1940

Burlington is a city of about 26,000 people located in southeastern Iowa about 45 miles from the southern border of the state. It is situated on the wooded bluffs overlooking the Mississippi River. South of the city the river valley is comparatively narrow, but to the north it widens to include flat agricultural bottom lands. Numerous wooded islands occur in the river within a radius of 5 to 10 miles north of the city. The trap was located in a residential district in the northern part of the town and was situated on the edge of the bluff among dense shrubbery and trees. The breeding areas which produce most of the mosquitoes affecting this city are in the valley and on the islands in the river.

The trap was operated for only 19 days, 13 of which were in June and 6 in July. Such a limited number of trap-nights are insufficient to show a seasonal record or an adequate account of the numbers and species which occurred. The trap records for the 19 nights, however, are included in Table 7.

## TRAP RECORDS FROM AMES, 1940

Ames has a population of about 12,000 and is located near the center of the state. It is surrounded by gently rolling farm land and winding creeks. About a mile to the east is the Skunk River, and through the city toward the southeast runs Squaw Creek. Several smaller creeks flowing eastward enter Squaw Creek near the city. The banks of these are wooded and numerous low areas occur along their beds which become flooded during heavy rains. Other low areas are present in which rain water stands for considerable periods, but most of the mosquito breeding occurs on low areas flooded by the creeks. The trap was situated in the northwest part of the city on the campus of Iowa State College. It was within 1 mile of Squaw Creek, Clear Creek, and College Creek, and within 3 miles of Onion Creek and the Skunk River.

A summary of the catches taken on 124 trap-nights is given in Table 8. About 80 per cent of the entire catch were *Aedes*, of which 79 per cent were *A. vexans*, 17 per cent were *Culex*, and only 1 per cent *Anopheles*. The average nightly catch was 48 females, and on 30 per cent of the nights 24 or more were taken.

The seasonal record of catches between May 6 and September 28 is given on Figure 6. Four periods of rainfall are shown, but only one period of increased mosquito abundance was recorded. The precipitation peaks shown for May 21 and June 25 were due to short, heavy rains which fell on May 18 and on June 22 and 23. The peak on June 30 represents a 4-day rain and those from August 14 to 24, accumulations over a 15-day period. It is probable that the precipitation periods of both July and August were responsible for the increased mosquito period, which began about August 25 and reached a peak on September 17. Conditions were favorable for adult activity during this period except on September 10, 11, 12, and from the 25 to 28 when the mean temperatures fell below 60° F.

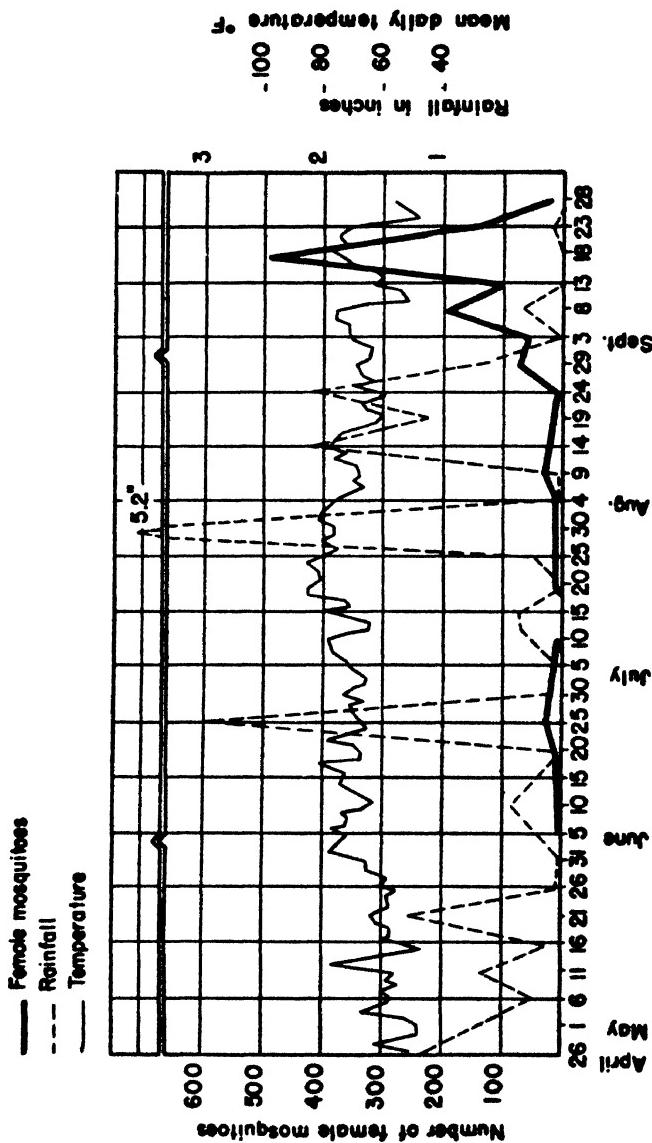


FIG. 6 Trap records, precipitation, and temperature for Ames, Iowa, 1940. Mosquito catches are averaged for 5-day periods, precipitation are totals for the same periods, and temperatures are daily means.

## TRAP RECORDS FROM DES MOINES, 1940

Des Moines, the capital city of Iowa, with a population of about 160,000, is located in the south central part of the state. It is situated at the junction of the Des Moines and Raccoon Rivers, part of the city being in the river valleys and part on the rolling wooded hills to the south and west. The trap was located in the north central residential district near Union Park, in a shaded garden containing numerous shrubs, trees, and flowers. The Capitol grounds and main business district were within a 2-mile radius, the State Fair grounds within a 3-mile radius, and Greenwood Park within a 4-mile radius of the trap. Aside from small rain pools, the heaviest breeding areas were along the Des Moines and Raccoon Rivers in northwest, southeast, and southern directions from Union Park.

The trap catches for 103 trap nights are summarized in Table 9. *A. vexans* were quite abundant in June, July, and August, and considerably so in September; *A. trivittatus* was caught fairly often in August and September. The record of *A. dupreei* is new and the only one from the state. *Aedes* made up 90 per cent of the total catch, *Culex* 8 per cent, and *Anopheles* 1 per cent. The average nightly catch was 100 females, and on 73 per cent of the nights 24 or more were taken.

The seasonal record of catches from May 30 to September 8 is shown in Figure 7. There were six principal periods of precipitation. The peak of May 21 represents a rather heavy 1-day rain, that of June 25, small rains over a period of 3 days, and that of July 10 to 15, two rains of one day each. The July 30 peak is for a 5-day rainy period, that of August 14,

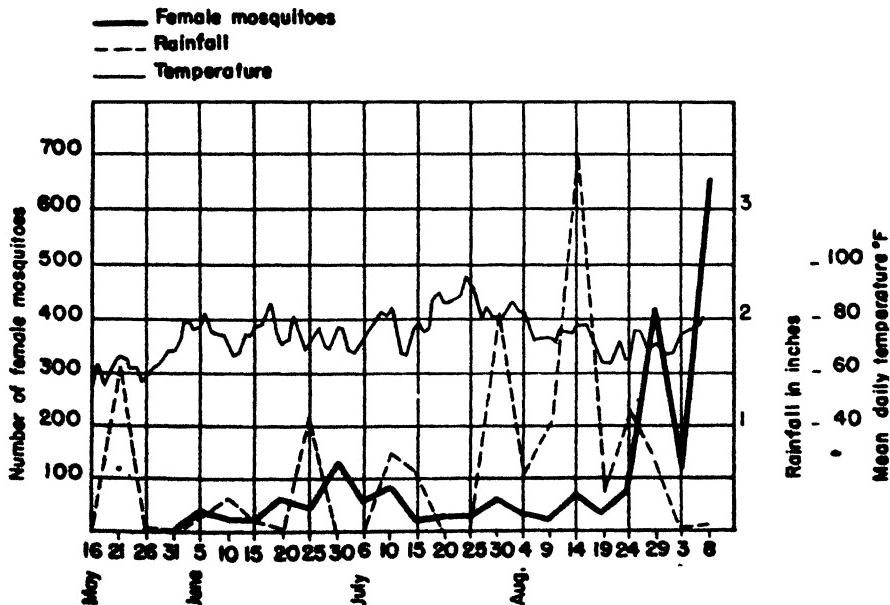


FIG. 7. Trap records, precipitation, and temperatures for Des Moines, Iowa, 1940. Mosquito catches are averaged for 5-day periods, precipitation are totals for the same periods, and temperatures are daily means.

TABLE 9  
TRAP RECORDS FOR DES MOINES, IOWA. 1940 (FEMALES)

	No. NIGHTS TRAP	Operated	AEDES	ANOPHELES	CULEX	OTHERS	TOTAL		% ANOPHELES	% AEDES	No. NIGHTS Col.	EXCEDED 24†	% WHICH HUMANS	BITE HUMANS
							No. NIGHTLY CATCH	% CATCH						
May.....	3													
June.....	29													
July.....	31													
Aug.....	31													
Sept.....	9													
Total.....	103													
Total males.....	1													
Dorsalis														
Duprecti														
Nigromaculata														
Trivittatus														
Vexans														
Punctipennis														
quadrimaculatus														
Apicalis*														
Cerraticulus														
Pictipennis														
Restuans														
Salinarius														
Tarsalis														
P. ciliata														
P. columbiæ														
P. feerox														
P. horrida														
T. inornata*														
U. sapphirina*														
Damaged														

\*Species which rarely bite humans.

†Not including species marked with asterisk.

a 6-day rain, and that of August 24 to 29, a 4-day rain. Two periods of increased mosquito abundance are shown, both of which were largely *A. vexans*. The first, represented by a short peak on June 30, began about June 20 and lasted until July 7 with the largest catch on June 29. It was started by the rains which began on June 21. The second one, much longer and of greater density than the first, started about August 12 and was at its peak on about September 8. During the first part of this period, August 12 to 24, adult activity was restrained by heavy rains and lower temperatures (minimums from August 18 to 24 below 60° F.). The lower catch average for the 5-day period ending September 3 can partially be explained by the lower temperatures of August 30 to September 2 (minimums below 60° F.).

#### TRAP RECORDS FROM COUNCIL BLUFFS, 1940

Council Bluffs, a city of 43,000 people, is in the southwestern section of Iowa in the flat valley of the Missouri River. The valley in this vicinity varies in width from 4 to 8 miles and is largely drained agricultural land. Dense tree growths, so characteristic of river valleys in the eastern part of the state, do not occur. The trap was located near the western residential district and park area at the river pumping station of the city waterworks department. It was situated in a well-kept garden of lawns, shrubs, and hedges. With respect to the trap, various parts of the city were located as follows: Dodge Park, 1 mile south; Lakeview Park, 2 miles northeast; main business district, 3 miles east; Fairmount Park, 3.5 miles southeast; Lake Manawa, 5 miles southeast; and the Carr's Lake-Mosquito Creek area, 8 miles southeast. Considerable *Culex* breeding occurred throughout most of the season in Big Lake (Lakeview Park) and in Carr's Lake. *Aedes* breeding was quite heavy in the Carr's Lake-Mosquito Creek area in June.

Table 10 shows the total catch for 99 trap-nights. The mosquito density was evidently very low throughout the entire period. *C. tarsalis* and *A. vexans* made up the bulk of the catches, being taken in about equal numbers. *Culex*, however, comprised 66 per cent of the total catch, *Aedes*, 30 per cent, and *Anopheles*, 1 per cent. The average nightly catch was only 7 females, and on only 2 nights did they exceed 24.

Seasonal records, Figure 8, show no 5-day periods for which the average catch was greater than 15 females. The low catches for the season up to July 25 are explained by the nature of the rainfall which was scattered and did not exceed 0.6 inches for any one day. The rains in late July and August did not occur over periods longer than 3 consecutive days and were, with the exception of August 11 and 25, less than 0.65 inches for any one day. This may partially account for the low catches in August.

#### TRAP RECORDS FROM SIOUX CITY, 1940

Sioux City is located on the banks of the Missouri River in the west central part of Iowa. It is a city of about 82,000 people, situated at the junction of the Floyd and Missouri River valleys. The eastern and western

TABLE 10  
TRAP RECORDS FOR COUNCIL BLUFFS, IOWA, 1940 (FEMALES)

\*Species which rarely bite humans.

† Not including species marked with asterisk.

TABLE 11  
Trap Records for Sioux City, Iowa, 1940 (Females)

	No. NIGHTS TRAP	OPERATED	AEDES	ANOPHELES	CULEX	OTHERS	TOTAL			AV. NIGHTLY CATCH	No. NIGHTS COL.	EXCERDED 24†	% ANOPHELES	% CULEX	% WHICH HABITUALLY	BITE HUMANS
							% AEDES	% ANOPHELES	% CULEX							
May.....	2						2	1	2	158	11	-	0	0	100	100
June.....	14						30	64	15	3	28	5	25	0	63	97
July.....	8						1	4	1	177	22	5	62	0	18	100
Aug.....	27						1	1	9	124	5	0	60	1	22	100
Total.....	51						14	1	1	462	9	6	48	0.3	35	99
Total males.....							9	11	...	230	5	...	91	0	7	...

\*Species which rarely bite humans.

†Not including species marked with asterisk.

residential districts are on the bluffs overlooking the river. About 3 miles west of the main business district and situated in the narrow Big Sioux River valley is a large wooded park and playground area, which is maintained by the city. The trap was operated in this park. Several small areas in the park and on the fair grounds south of the park become flooded during rainy periods and serve as *Aedes* breeding places.

The catches for 51 trap-nights are summarized in Table 11. These nights were scattered over a period of more than 3 months and are, therefore, considered inadequate to express the numbers or seasonal occur-

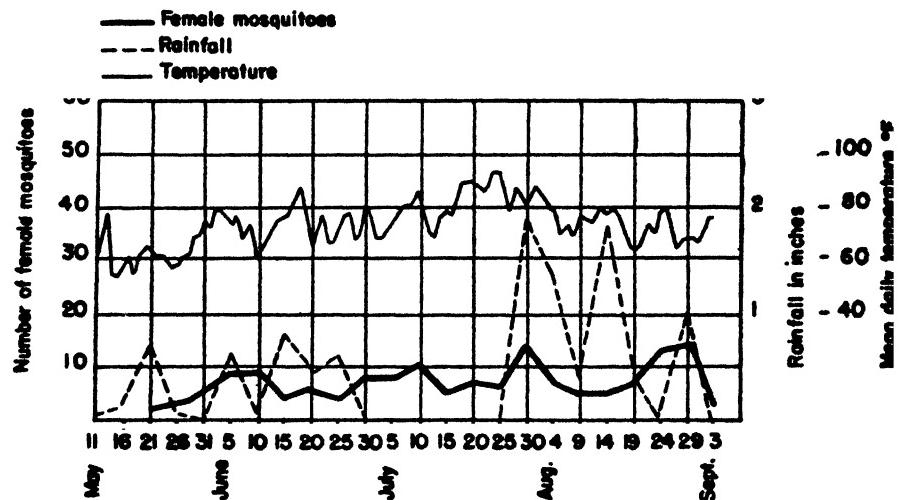


FIG. 8. Trap records, precipitation, and temperatures for Council Bluffs, Iowa, 1940. Mosquito catches are averaged for 5-day periods, precipitation are totals for the same periods, and temperatures are daily means.

rence of mosquitoes which may have occurred in the vicinity. For the 51 nights of operation the average nightly catch was 9 females and on only 12 per cent of the nights, 24 or more were taken. *Aedes* made up 48 per cent of the total catch, *Culex*, 35 per cent, and *Anopheles*, 0.3 per cent.

#### TRAP RECORDS FROM RUTHVEN, 1940

Ruthven is a rural community of about 900 people located in northwest Iowa. It is surrounded by flat to somewhat undulating farm land. Several small lakes lying within a radius of 10 miles are situated south and northwest of the town. About 5 miles northwest of town, Lost Island Lake, Mud Lake, Round Lake, and Trumbull Lake serve as fishing and hunting grounds for many sportsmen; numerous summer cottages are used by vacationists from many parts of Iowa. In general, this trap area is typical of the Iowa lake region in Dickinson, Emmet, Clay, and Palo Alto Counties. Most of the lakes are shallow, and the permanent or intermit-

tent marshes lying between them cover many acres. The trap was situated on the west shore of Lost Island Lake about 4 miles northwest of Ruthven. Extensive mosquito breeding areas were on all sides and within a 5-mile radius.

The summary of trap catches for 85 nights is given in Table 12. The predominant species was *A. vexans*, but numerous *C. tarsalis* were also taken. The record of *T. impatiens* is the only one from the state. *Aedes* made up 55 per cent of the total catch, *Culex*, 43 per cent, and *Anopheles*

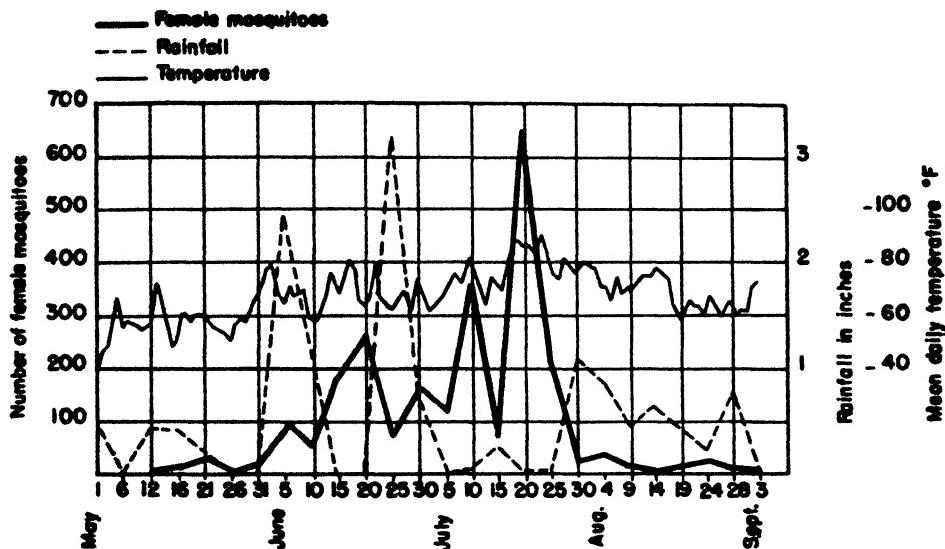


FIG. 9. Trap records, precipitation, and temperatures for Ruthven, Iowa, 1940. Mosquito catches are averaged for 5-day periods, precipitation are totals for the same periods, and temperatures are daily means.

(chiefly *walkeri*), about 1 per cent. The average nightly catch was 110, and on 47 per cent of the nights 24 or more females were taken.

Figure 9 shows that most of the rainfall for this area (precipitation and temperature records are for Spencer, Iowa) occurred between June 4 and July 29, with heaviest rains on June 4, 21, and 23, and July 29. Those of May and August were small and scattered. There was a general period of increased mosquito abundance from June 12 to July 23, with prominent peaks on June 16, July 8, and July 20. These were due to *A. vexans* and *C. tarsalis*. During the peak periods conditions were generally favorable for flight, and during those between peak catches rain and lower temperatures occurred.

#### SUMMARY

The New Jersey mosquito trap was used during the breeding season of 1940 to sample the composition and density of female mosquitoes in ten Iowa cities. Table 13 is a summary of the trap records taken during these investigations.

TABLE 12  
Trap Records for Ruthven, Iowa, 1940 (FEMALES)

AEDES	ANOPHELES	CULEX	OTHERS	No. NIGHTS TRAP		OPERATOR	No. NIGHTS COL.	EXCERPTS 24+	% ANOPHELES	% CULEX	% AEDES	AV. NIGHTLY CATCH	No. NIGHTS COL.	EXCERPTS 24+	% ANOPHELES	% CULEX	BITE HUMANS	
				May	June													
				17	3	46	2	79	2	88	229	13	2	24	0	36	65	
				22	10	2,345	1	676	1	4	3,160	144	15	75	0,3	23	98	
				26	2	1	2,626	1	2,727	2	1	5,601	215	20	47	1	52	99
				20	...	...	48	...	41	...	319	16	3	15	0	84	98	
				Total														
				85	3	17	10	1	5,065	1	1	3,707	2	1	6	168	9	9,309
				Total males	...	8	61	39	...	9,349	...	2	10	5	63	1,299	1	89
																129	...	13

\*Species which rarely bite humans.

†Not including species marked with asterisk.

TABLE 13  
SUMMARY OF TRAP RECORDS FOR 10 IOWA CITIES, 1940

Locality	No. NIGHTS Trap Operated	ANOPHELES		CULEX		Total males
		VEXANS	quadrimaculatus	walkei	apicalis*	
Ames	124	39	4,657	57	6	21
Burlington	19	1	285	7	17	242
Council Bluff	99	10	1	202	6	56
Davenport	90	8	1	3	2	99
Des Moines	103	1	12	84	18	106
Dubuque	49	1	1	1,494	26	142
Lansing	77	1	2	9	2,707	360
Muscatine	86	3	17	10	40	360
Ruthven	85	3	14	1	17	906
Sioux City	51	1	1	15	1	71
Total	783	3	26	1	183	3,684
Total males	.....	8	64	69	1	144
					14	11
					165	2,066
					7	677
					165	3,410
					7	7,210
					805	2,042
						5,990

\*Species which rarely bite humans.

†Not including species marked with asterisk.

TABLE 13—(Continued)

Locality	No. NIGHTS TRAP	OPERATORS	Psorophora	Others	TOTAL			% ANOPHLES	% CULEX	% WHICH HABITUALLY BITE HUMANS
					M. perturbans	O. signifera*	T. imortata*	C. spp. <i>hirtima</i> *	Damaged	
Amer.	124	7	36	37	17	15	15	5,906	48	99
Burlington	19	...	3	1	3	5	5	427	23	27
Council Bluffs	99	...	7	8	12	12	7	718	2	30
Davenport	90	1	15	2	60	199	7	8,829	66	59
De Moines	103	10	2	2	8	46	25	10,371	100	75
Dakque	49	...	...	...	7	156	256	4,460	91	34
Lansing	77	1	...	...	2	10	2	3,135	41	20
Muscatine	86	...	...	28	1	16	2	2,582	30	43
Ruthven	85	...	1	2	168	6	9	9,309	110	40
Sioux City	51	1	...	44	3	...	...	462	9	6
Total	783	19	3	1	2	88	48	1	243	6
Total males	...	...	2	4	1	5	51	...	176	..
							147	920	41,497	53
									59	331
									66	67
									0.2	1
									31	29
										98

\*Species which rarely bite humans.

†Not including species marked with asterisk.

Of the total females taken in the traps, 67 per cent were *Aedes*, 99 per cent of these being *A. vexans*; 29 per cent were *Culex* and about 1 per cent were *Anopheles*. With the exception of two traps, Council Bluffs and Dubuque, catches of *Aedes* were higher than those of *Culex*.

Average nightly catches ranged from 7 at Council Bluffs to 110 at Ruthven. These catches were considerably higher for the traps along the Mississippi River than for those along the Missouri River.

The percentages of trap nights during which 24 females were taken are given as follows: Davenport, 73; Des Moines, 73; Dubuque, 64; Muscatine, 50; Ruthven, 47; Burlington, 37; Ames, 30; Lansing, 24; Sioux City, 12; and Council Bluffs, 2.

The data on seasonal occurrence indicate that periods of increased mosquito abundance follow within 10 to 20 days after periods of increased precipitation. Higher populations occurred following prolonged periods of heavy rains.

The mean temperatures were relatively high on nights when the larger catches were taken, and during periods of increased abundance; the smaller catches were generally on nights with lower means.

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# EVALUATION OF GERMICIDAL PROPERTIES OF SODIUM HYDROXIDE AND ALKALINE WASHING COMPOUNDS\*

C. R. ARNOLD† AND MAX LEVINE‡

From the Laboratories of the Department of Bacteriology and Industrial Science Research Institute, Iowa State College

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For a number of years national, state, and local sanitary agencies have been active in setting up standards for mechanical bottle washing in beverage, dairy, and other food industries. The standards proposed and established usually specify (1) a minimum time of exposure of the bottles, (2) a stipulated minimum concentration of alkali or caustic solution, which is (3) maintained at a designated minimum temperature. With very few exceptions these standards are precise and inflexible, since there has not been available a suitable method for converting a given standard as to time of exposure, temperature, and concentration of alkali into one of equivalent sterilizing efficiency, if any of these factors was varied. Hence, a mathematical expression was sought which could be utilized to evaluate the germicidal properties of alkalies for various combinations of these three factors.

Solutions employed in mechanical bottle washers, such as are used by the dairy and beverage industries, generally contain sodium hydroxide to which has been added milder alkalies such as sodium carbonate, tri-sodium phosphate, or small amounts of alkaline silicates. The germicidal properties of these mixtures, as determined by various workers using *Bacillus metiens*, have been found to depend primarily upon the concentration of NaOH; however, the milder alkalies present, such as  $\text{Na}_2\text{CO}_3$ ,  $\text{Na}_3\text{PO}_4$ , etc., not only act as detergents and water softeners but materially increase the germicidal properties of the solution (Levine, Buchanan, and Toulouse,<sup>1</sup> Lowman, Buchanan, and Levine<sup>2</sup>).

The germicidal properties of sodium hydroxide were first systematically investigated by Levine, Buchanan, and Lease<sup>3</sup> when they determined the effect of different concentrations of NaOH at various temperatures on spores of *Bacillus metiens*. Using the same organism, Levine, Peterson,

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† Formerly Fellow American Bottlers of Carbonated Beverages.

‡ Professor in Charge of A.B.C.B. Fellowship at Iowa State College.

<sup>1</sup> Levine, Max, J. H. Buchanan, and J. H. Toulouse. Influence of sodium chloride, sodium carbonate, and tri-sodium phosphate on germicidal efficiency of sodium hydroxide. *Iowa State Coll. Jour. Sci.* 2: 19-29. 1927.

<sup>2</sup> Lowman, O. E., J. H. Buchanan, and Max Levine. Effects of salts on alkali disinfection. *Iowa State Coll. Jour. Sci.* 5: 251-68. 1931.

<sup>3</sup> Levine, Max, J. H. Buchanan, and Grace Lease. Effect of concentration and temperature on the germicidal efficiency of sodium hydroxide. *Iowa State Coll. Jour. Sci.* 1: 379-84. 1927.

and Buchanan,<sup>4</sup> and Myers<sup>5</sup> studied the effect of NaOH, Na<sub>2</sub>CO<sub>3</sub> and Na<sub>3</sub>PO<sub>4</sub>, 10 H<sub>2</sub>O and mixtures of NaOH and sodium carbonate or sodium phosphate held at stipulated hydrogen-ion concentrations, thereby demonstrating that the OH-ion concentration was not in itself a direct measure of germicidal efficiency for various alkalis or mixtures.

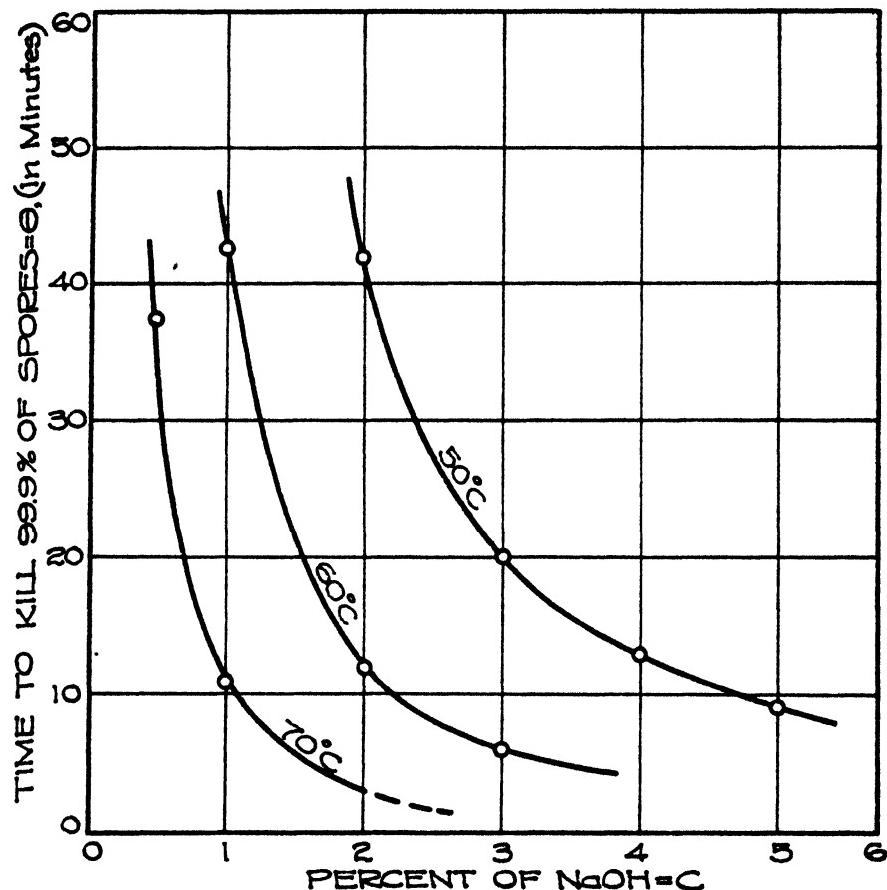


FIG. 1 RELATION OF CONCENTRATION  
OF NaOH TO KILLING TIME

The spores of this organism were found not to die off logarithmically, i.e., at a rate constantly proportional to the number of viable organisms present at any given time, but rather at increasing rates as the period of exposure increased. The killing time (specifically, the time necessary

<sup>4</sup> Levine, Max, E. E. Peterson, and J. H. Buchanan. Germicidal efficiency of sodium hydroxide, sodium carbonate, and tri-sodium phosphate at the same H-ion concentration. *Ind. Engr. Chem.* 19: 1338-40. 1927.

<sup>5</sup> Myers, Robert P. The germicidal properties of alkaline washing solutions, with special reference to the influence of hydroxyl-ion concentration, buffer index, and osmotic pressure. *Jour. Agr. Research* 38: 521-63. 1929.

to kill 99.9 per cent of the viable spores of *Bacillus metiens*) was selected as a suitable measure of germicidal power.

In Table 1 are given the times to kill 99.9 per cent of the spores of *Bacillus metiens* by various concentrations of sodium hydroxide at temperatures of 50°C., 60°C., and 70°C. (after Levine, Buchanan, and Lease, 1927). When these killing times are plotted against the concentration of NaOH, curves, which on inspection appear to be characteristically logarithmic, are obtained as shown in Figure 1. When the logarithms of the killing times are plotted against the logarithms of the concentrations of sodium hydroxide, straight lines are obtained as in Figure 2. The equa-

TABLE 1  
TIME TO KILL 99.9% OF SPORES OF *Bacillus metiens* WITH NaOH

PERCENTAGE NaOH	TEMPERATURE		
	50°C.	60°C.	70°C.
	Killing Time in Minutes		
0.5.....	.....	.....	37.4
1.0.....	.....	42.5	10.7
2.0.....	41.7	11.7	.....
3.0.....	19.8	5.7	.....
4.0.....	12.4	.....	.....
5.0.....	8.2	.....	.....

tions of these lines as determined by the method of least squares, were found to be

$$(50^\circ\text{C.}) \quad \log \theta = 2.1471 - 1.7627 \log C: \quad (1)$$

$$(60^\circ\text{C.}) \quad \log \theta = 1.6690 - 1.7924 \log C: \quad (2)$$

$$(70^\circ\text{C.}) \quad \log \theta = 1.0292 - 1.8060 \log C: \quad (3)$$

where " $\theta$ " is the killing time in minutes, and "C" is the concentration of NaOH in percentage.

It appears from Figure 2 that the lines are parallel and almost equidistant from each other. This indicates a probable continuous relationship among the three variables—killing time, temperature, and concentration. As a matter of fact, the following equation may be derived by the method of least squares, considering the killing time as a function of both the temperature and the concentration:

$$\log \theta = 4.9815 - 1.7912 \log C - 0.05630 t, \quad (4)$$

where " $\theta$ " is the killing time in minutes, "C" the concentration of NaOH in

percentage, and "t" the temperature in degrees centigrade. When the temperature is measured in degrees Fahrenheit the equation is:

$$\log \theta = 5.9840 - 1.7912 \log C - 0.03129 t \quad (5)$$

The suitability of these equations to represent the information in Table 1 and Figures 1 and 2 is brought out in Table 2, in which are compared the experimental and calculated values of the killing times for various temperatures and concentrations of sodium hydroxide. The

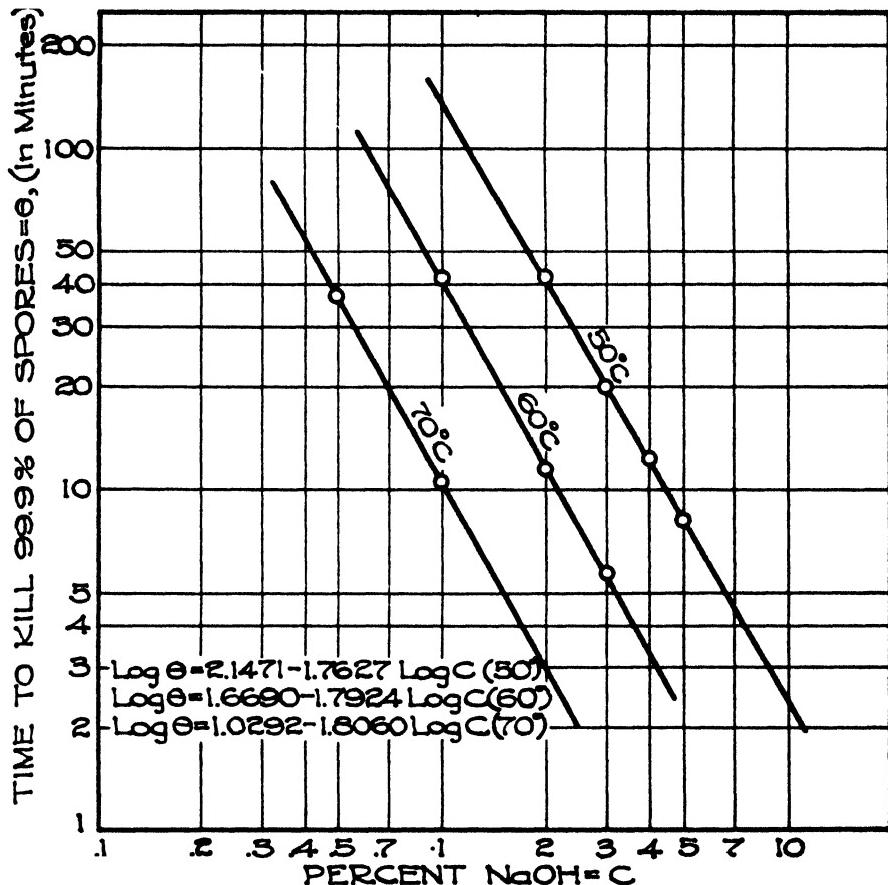


FIG. 2 RELATION OF CONCENTRATION OF NaOH TO KILLING TIME

deviations of the calculated from the experimental values of the killing times are slight, the maximum difference being 5.6 per cent but generally less than 2 per cent, or well within the limits of experimental errors associated with plate counts.

Equation (4) is an equation of a plane, a projection of a portion of which, when drawn on paper, would give a perspective similar to Figure 3. It may be pointed out that equations (1), (2), and (3) are equations for

the lines made at the intersections of plane a-b-c-d-e in Figure 3 described by equation (4), with planes which pass through the temperature axis at 50°C., 60°C., and 70°C., respectively, and are parallel to the plane a-b-k-n-m. It should be noted that, if the intersections of the concentration-killing time planes at 50°C., 60°C., and 70°C., with plane a-b-c-d-e were projected on the concentration-killing time plane a-b-k-n-m, Figure 2 would be obtained.

The interpretation of the constants in equation (4) are based on previous work in this laboratory as well as on the literature concerned

TABLE 2  
COMPARISON OF EXPERIMENTAL AND CALCULATED KILLING TIMES FOR *Bacillus metiens*  
SPORES BY SODIUM HYDROXIDE

% NaOH	50°C.			60°C.			70°C.		
	Exptl.	Calc. *	% Diff.	Exptl.	Calc. *	% Diff.	Exptl.	Calc. *	% Diff.
0.5	.....	.....	.....	.....	.....	.....	37.4	38.0	+1.1
1.0	.....	.....	.....	42.5	40.1	-5.6	10.7	11.0	+2.8
2.0	41.7	42.4	+1.6	11.7	11.6	-0.9	.....	.....	.....
3.0	19.8	20.5	+3.5	5.7	5.7	0.0	.....	.....	.....
4.0	12.4	12.3	-0.8	.....	.....	.....	.....	.....	.....
5.0	8.2	8.2	0.0	.....	.....	.....	.....	.....	.....

$$^*\log \theta = 4.9815 - 1.7912 \log C - 0.0563 t.$$

with death rates of bacteria. Let equation (4) or (5) be written as follows:

$$\log \theta = K - a \log C - bt \quad (6)$$

This may be employed as a general equation for evaluating the resistance of an organism or the activity of a germicide. For equation (6), the constant "K" and coefficients "a" and "b" may be determined experimentally for any germicide and organism by ascertaining the killing times at various temperatures and concentrations.

Phelps<sup>6</sup> reported that the concentration and temperature coefficients apparently do not vary with the test organism. Accepting this concept, "a" and "b" in equation (6) are factors associated with the effect of concentration and temperature, respectively, on the germicidal efficiencies of the disinfectant under consideration. These coefficients are therefore considered characteristic for a given germicide and are presumed to be independent of the test organism. The effect of temperature alone on the spores of *Bacillus metiens* was found to be practically nil at 50°C., 60°C., and 70°C. Therefore "b" is considered to be associated with the effect of temperature on efficiency of the germicide.

<sup>6</sup> Phelps, Earle B. The application of certain laws of physical chemistry to the standardization of disinfectants. Jour. Inf. Dis. 8: 27-38. 1911.

"K" in equation (6) is a constant characteristic for the resistance of a test organism against a test disinfectant. Thus, for a given germicide,

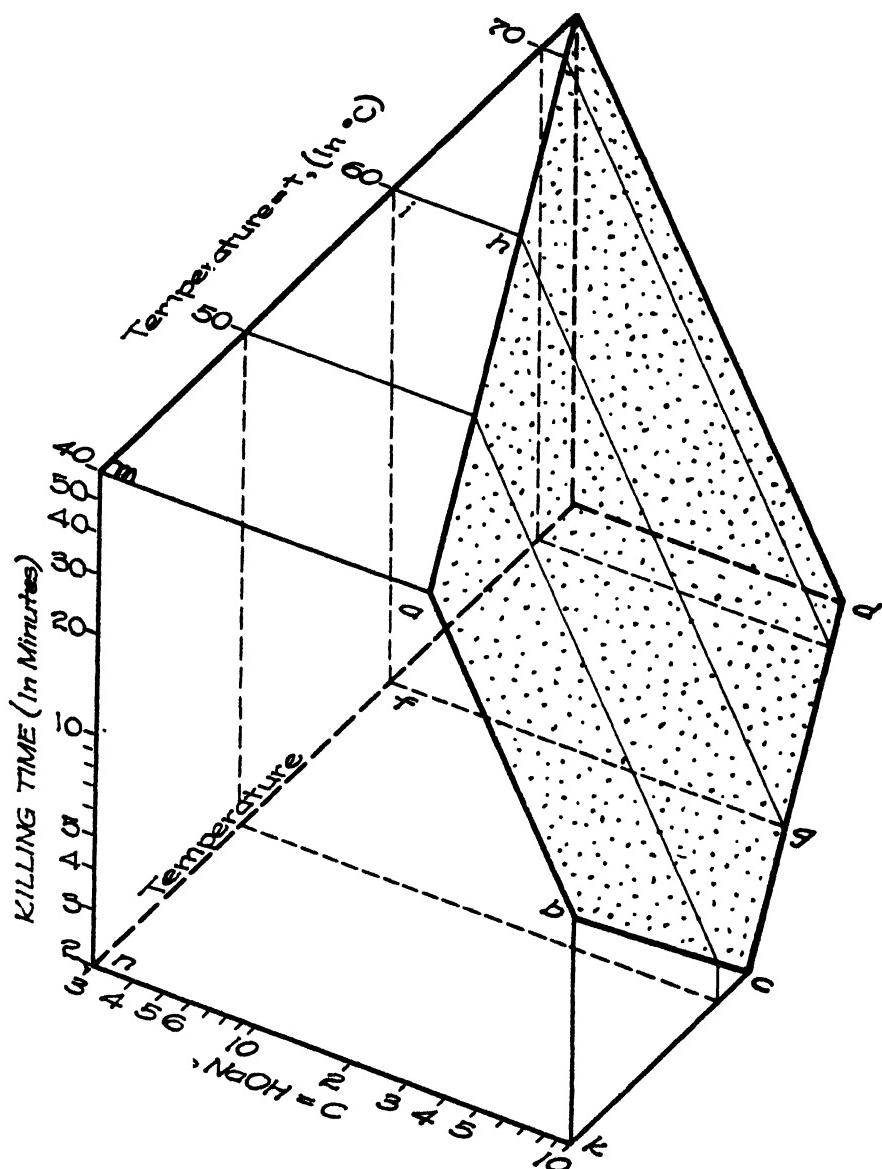


FIG. 3 RELATION OF KILLING TIME TO CONCENTRATION AND TEMPERATURE

a large value of "K" signifies that the organism is very resistant. Conversely, a low value indicates little resistance. Moreover, when standards for bottle washing are stipulated by law there has in effect been established

a standard of germicidal efficiency adequate to destroy a hypothetical organism the relative resistance of which, with respect to other organisms, is measured by the value of "K".

The foregoing explanation of the significance of the constants in equation (6) suggests several possible uses for the equation. It has been previously mentioned that there is need for a suitable method by which a given standard (which stipulates a time, temperature, and concentration of alkali) for bottle washing may be accurately and conveniently converted into one of equivalent germicidal efficiency if some other time, concentration, or temperature than that specified is employed. Such equivalents may then be utilized when the specified standard is difficult or impossible to meet, either because of the design of the washing machine or poor economy of operation under the specified standard.

In order to derive equivalents for a bottle washing standard, it is necessary merely to substitute in equation (6) the concentration of caustic, the temperature, and the holding time (the time during which the bottle is subjected to the washing solution) recommended, and solve for "K", using  $a = 1.7912$  and  $b = 0.0563$ , the values characteristic for NaOH as a germicide (see equation (4) or (5), where " $b$ " = 0.03129 when temperature is in degrees Fahrenheit). Having thus determined "K" it is then possible to ascertain various combinations of time, temperature, and concentration of NaOH which will have the same germicidal efficiency as the standard in question.

#### GERMICIDAL EQUIVALENTS FOR NEW YORK CITY MILK BOTTLE WASHING STANDARD

For example, the New York City Health Department stipulates a minimum holding time of 7 minutes, a temperature of 150°F. (65.56°C.), and a concentration of 2 per cent sodium hydroxide for washing milk bottles. By substituting these values in equation (6) and solving for "K" it is found that

$$K_{(N.Y.C.)} = \log 7 + 1.7912 \log 2 + 0.0563 \times 65.56 = 5.0753 \quad (7)$$

This number is characteristic for a hypothetical organism which is killed in 7 minutes by a 2 per cent sodium hydroxide solution, a concentration of 2 per cent at a temperature of 65.56°C. (150°F.). Having found "K"<sub>(N.Y.C.)</sub> it is a simple matter to determine the germicidal sterilizing equivalents for any desired combination of killing time, concentration of NaOH, and temperature by substituting 5.0753 for "K", 1.7912 for "a", and 0.0563 for "b" in equation (6) thus:

$$\log \theta = 5.0753 - 1.7912 \log C - 0.05630 t \quad (8)$$

The above equation holds only when the temperature "t" is measured in degrees centigrade. When the Fahrenheit scale is employed the following equation must be utilized to determine germicidal equivalent for the New York City standard:

$$\log \theta = 6.0777 - 1.7912 \log C - 0.03129 t \quad (9)$$

TABLE 3  
GERMICIDAL EQUIVALENTS

(Based on New York City Specifications for Milk Bottles)  
*Concentrations for Designated Temperatures and Exposure Period\**

TIME (IN MIN.)	TEMPERATURE (°F.)													
	Concentration of NaOH (in %)													
	175	170	165	160	155	150	145	140	135	130	125	120	115	110
1.....	2.2	2.6	3.2	4.0	5.9	7.2	8.9	10.8	13.2	16.2	19.8	24.2	29.6	
2.....	1.5	1.8	2.2	2.7	3.3	4.0	4.9	6.0	9.0	10.9	13.5	16.4	20.1	
3.....	1.2	1.4	1.8	2.1	2.6	3.2	3.9	4.8	5.9	7.2	8.8	10.7	13.1	
4.....	1.0	1.2	1.5	1.8	2.2	2.7	3.3	4.1	5.0	6.2	7.5	9.1	11.1	
5.....	0.9	1.1	1.3	1.6	2.0	2.4	2.9	3.6	4.4	5.4	6.6	8.1	9.9	
6.....	0.8	1.0	1.2	1.5	1.8	2.2	2.7	3.3	4.0	4.9	6.0	7.3	8.9	
7.....	0.7	0.9	1.1	1.3	1.6	2.0	2.4	3.0	3.6	4.5	5.5	6.7	8.2	
8.....	0.7	0.8	1.0	1.2	1.5	1.9	2.3	2.8	3.4	4.2	5.1	6.2	7.6	
9.....	0.6	0.8	1.0	1.2	1.4	1.7	2.1	2.6	3.2	3.9	4.7	5.8	7.1	
10.....	0.6	0.7	0.9	1.1	1.3	1.6	2.0	2.4	3.0	3.7	4.8	5.5	6.7	
11.....	0.6	0.7	0.8	1.0	1.2	1.5	1.9	2.3	2.8	3.5	4.2	5.2	6.4	
12.....	0.5	0.7	0.8	1.0	1.2	1.5	1.8	2.2	2.6	3.3	4.0	4.9	6.0	
13.....	0.5	0.6	0.8	0.9	1.2	1.4	1.7	2.1	2.6	3.2	3.9	4.7	5.8	
14.....	0.5	0.6	0.7	0.9	1.1	1.4	1.7	2.0	2.5	3.0	3.7	4.5	5.5	
15.....	0.5	0.6	0.7	0.9	1.1	1.3	1.6	2.0	2.4	2.9	3.6	4.4	5.3	

$$\log C = 3.3931 - 0.55828 \log \theta - 0.01747 t$$

$C = \% \text{ NaOH}$ ;  $\theta = \text{killing time in minutes}$ ;  $t = \text{temperature in } ^\circ\text{F.}$

\*"Exposure Period" = "Killing Time" in experimental determinations.

By means of equation (9) a table may be prepared to show the various holding periods, or times in minutes required for any stipulated temperature and concentration of caustic. A more useful and practical table, however, would be one in which the concentration of caustic is the variable while the holding times and temperatures are stipulated. Solving equation (9) for "log C" gives the following:

$$\log C = 3.3931 - 0.55828 \log \theta - 0.01747 t \quad (9a)$$

Equation (9a) was employed in development of Table 3 for germicidal equivalents of the New York City milk bottle washing standards.

#### GERMICIDAL EQUIVALENTS FOR CITY OF CHICAGO MILK BOTTLE WASHING STANDARDS

The City of Chicago Health Department for a number of years has required that milk bottles be subjected to 1.6 per cent NaOH at a temperature of 120°F. for 5 minutes. Proceeding as was described for the New York City standard  $K_{(Chicago)}$  is found to have a value of 3.8171 if the temperature is expressed in °C. or 4.8195 when using °F., the equations for germicidal equivalents being

$$\log \theta = 3.8171 - 1.7912 \log C - 0.05630 t, \text{ when } t = ^\circ\text{C., and}$$

$$\log \theta = 4.8195 - 1.7912 \log C - 0.03129 t, \text{ when } t = ^\circ\text{F.,}$$

and solving the latter for "log C" gives equation

$$\log C = 2.6906 - 0.55828 \log \theta - 0.01747 t \quad (9b)$$

which was employed in development of Table 4.

#### GERMICIDAL EQUIVALENTS FOR CALIFORNIA SPECIFICATIONS FOR WASHING GLASS AND CROCKERY FOOD CONTAINERS

The food officials of California have stipulated that refillable glass food containers be subjected to 2.5 per cent caustic, at a temperature of 120°F. for 5 minutes. By substitution of these values in equations (4) and (5) the  $K_{(California)}$  values obtained are 4.1646 when temperature is expressed in °C. and 5.1670 when temperature is in °F., the respective equations being

$$\log \theta = 4.1646 - 1.7912 \log C - 0.05630 t, \text{ (when } t = ^\circ\text{C.)}$$

$$\log \theta = 5.1670 - 1.7912 \log C - 0.03129 t, \text{ (when } t = ^\circ\text{F.),}$$

and solving the latter for "log C" gives the equation

$$\log C = 2.8843 - 0.55828 \log \theta - 0.01747 t \quad (9c),$$

which was employed for computation of Table 5.

#### GERMICIDAL EQUIVALENTS FOR AMERICAN BOTTLERS OF CARBONATED BEVERAGES BOTTLE WASHING STANDARD

The method for deriving the equation to be used in calculating the germicidal equivalents for the American Bottlers of Carbonated Beverages Standard for washing beverage bottles is somewhat more compli-

TABLE 4  
GERMICIDAL EQUIVALENTS  
(Based on Chicago Specifications for Milk Bottles)  
*Concentrations for Designated Temperatures and Exposure Period\**

TIME (IN MIN.)	TEMPERATURE (°F.)													
	Concentration of NaOH (in %)													
175	170	165	160	155	150	145	140	135	130	125	120	115	110	
1.....	0.45	0.55	0.65	0.80	0.95	1.20	1.45	1.75	2.15	2.65	3.20	3.95	4.80	5.87
2.....	0.30	0.35	0.45	0.55	0.65	0.80	1.00	1.20	1.45	1.80	2.20	2.65	3.25	3.99
3.....	0.25	0.30	0.35	0.45	0.55	0.65	0.80	0.95	1.15	1.40	1.75	2.15	2.60	3.18
4.....	0.20	0.25	0.30	0.35	0.45	0.55	0.65	0.80	1.00	1.20	1.50	1.80	2.20	2.71
5.....	0.20	0.20	0.25	0.30	0.40	0.50	0.60	0.70	0.85	1.05	1.30	1.60	1.95	2.39
6.....	0.15	0.20	0.25	0.30	0.40	0.50	0.55	0.65	0.80	0.95	1.20	1.45	1.75	2.16
7.....	0.15	0.20	0.20	0.25	0.30	0.40	0.50	0.60	0.70	0.90	1.10	1.30	1.60	1.98
8.....	0.10	0.15	0.20	0.25	0.30	0.35	0.45	0.55	0.65	0.80	1.00	1.25	1.50	1.84
9.....	0.10	0.15	0.20	0.20	0.30	0.35	0.40	0.50	0.60	0.75	0.95	1.15	1.40	1.72
10.....	0.10	0.15	0.16	0.20	0.25	0.30	0.40	0.50	0.60	0.70	0.95	1.10	1.30	1.62
11.....	0.10	0.15	0.15	0.20	0.25	0.30	0.35	0.45	0.55	0.70	0.85	1.05	1.25	1.54
12.....	0.10	0.15	0.15	0.15	0.20	0.25	0.30	0.35	0.45	0.55	0.65	0.80	1.00	1.20
13.....	0.10	0.10	0.15	0.20	0.25	0.30	0.25	0.40	0.50	0.60	0.75	0.95	1.15	1.40
14.....	0.10	0.10	0.10	0.15	0.20	0.25	0.30	0.35	0.40	0.50	0.60	0.75	0.90	1.10
15.....	0.10	0.10	0.10	0.15	0.15	0.15	0.20	0.25	0.30	0.40	0.45	0.55	0.70	0.85

$$\log C = 2.6906 - 0.55828 \log \theta - 0.01747 t$$

C = % NaOH;  $\theta$  = killing time in minutes;  $t$  = temperature in °F.

"Exposure Period" = "Killing Time" in experimental determinations.

TABLE 5  
GERMICIDAL EQUIVALENTS  
(Based on California Specifications for Glass and Crockery Food Containers)  
*Concentrations for Designated Temperatures and Exposure Periods\**

TIME (IN MIN.)	TEMPERATURE (°F.)													
	CONCENTRATION OF NaOH (IN %)													
	175	170	165	160	155	150	145	140	135	130	125	120	115	110
1.....	0.68	0.81	1.00	1.2	1.5	1.8	2.2	2.7	3.4	4.1	5.0	6.1	7.5	9.2
2.....	0.47	0.56	0.68	0.83	1.0	1.2	1.5	1.9	2.3	2.8	3.4	4.2	5.1	6.2
3.....	0.37	0.44	0.54	0.67	0.81	1.0	1.2	1.5	1.8	2.2	2.7	3.3	4.1	5.0
4.....	0.31	0.37	0.46	0.57	0.69	0.84	1.0	1.3	1.6	1.9	2.3	2.8	3.5	4.2
5.....	0.28	0.34	0.40	0.50	0.61	0.75	0.91	1.1	1.4	1.7	2.0	2.5	3.1	3.7
6.....	0.25	0.31	0.37	0.45	0.55	0.67	0.82	1.0	1.2	1.5	1.8	2.3	2.8	3.4
7.....	0.22	0.28	0.34	0.41	0.51	0.62	0.76	0.93	1.1	1.4	1.7	2.1	2.5	3.1
8.....	0.22	0.25	0.31	0.38	0.47	0.58	0.70	0.86	1.1	1.3	1.6	1.9	2.4	2.9
9.....	0.19	0.25	0.29	0.36	0.44	0.54	0.66	0.80	0.98	1.2	1.5	1.8	2.2	2.7
10.....	0.19	0.22	0.28	0.34	0.42	0.51	0.62	0.76	0.93	1.1	1.4	1.7	2.1	2.5
11.....	0.19	0.22	0.26	0.32	0.39	0.48	0.59	0.72	0.88	1.1	1.3	1.6	2.0	2.4
12.....	0.16	0.22	0.25	0.31	0.37	0.46	0.56	0.67	0.84	1.0	1.3	1.5	1.9	2.3
13.....	0.16	0.19	0.24	0.29	0.36	0.44	0.54	0.66	0.80	0.98	1.2	1.5	1.8	2.2
14.....	0.16	0.19	0.23	0.28	0.34	0.42	0.51	0.63	0.77	0.94	1.1	1.4	1.7	2.1
15.....	0.16	0.19	0.22	0.27	0.33	0.40	0.50	0.60	0.74	0.90	1.1	1.4	1.7	2.0

$$\log C = 2.8843 - 0.55828 \log \theta - 0.01747 t$$

C = % NaOH;  $\theta$  = killing time in minutes;  $t$  = temperature in °F.

\*"Exposure Period" = "Killing Time" in experimental determinations.

cated in that it is necessary to mathematically evaluate the effect of presence of the milder alkalies,  $\text{Na}_2\text{CO}_3$  and  $\text{Na}_3\text{PO}_4$ , with sodium hydroxide on the germicidal efficiency of the resulting solutions. It has been reported (Levine, Buchanan, and Toulouse<sup>1</sup>; Lowman, Buchanan, and Levine<sup>2</sup>; and Levine, Peterson, and Buchanan<sup>4,7</sup>) that the addition of  $\text{NaCl}$ ,  $\text{Na}_2\text{CO}_3$ , and  $\text{Na}_3\text{PO}_4$  markedly increases the germicidal properties of caustic soda.

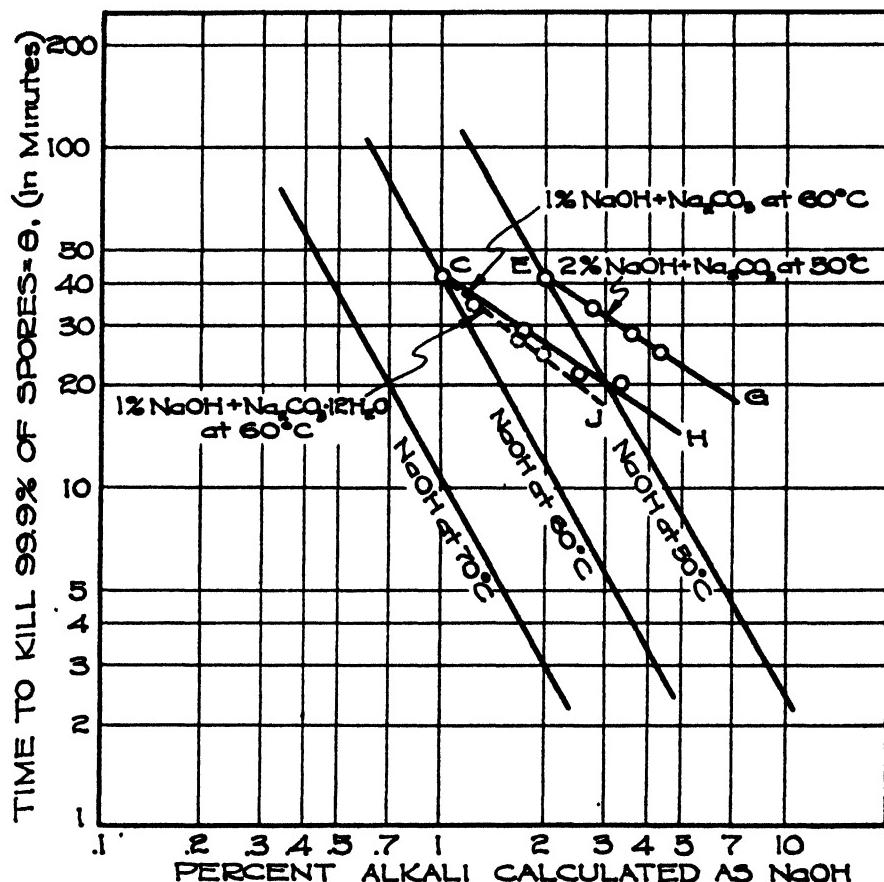


FIG. 4 RELATION OF CONCENTRATION OF ALKALI TO KILLING TIME

The magnitudes of the increased germicidal effect for mixtures of sodium hydroxide and sodium carbonate are given in Table 4.

It may be observed from Figure 4 that at 50°C. the killing times for various percentage concentrations of  $\text{NaOH}-\text{Na}_2\text{CO}_3$  mixtures fall along the straight line EG, intersecting at 2 per cent NaOH, the line which shows the effect of various concentrations of NaOH alone on the killing time.

<sup>1</sup> Levine, Max, E. E. Peterson, and J. H. Buchanan. Germicidal efficiency of sodium hydroxide and sodium hydroxide-carbonate mixtures at the same H-ion concentration. Ind. Engr. Chem. 20: 63-65. 1927.

Similarly, at 60°C. the killing times for various concentrations of NaOH-Na<sub>3</sub>CO<sub>3</sub> mixtures lie rather closely along the straight line, CH, which intersects the line denoting the effect of NaOH alone on the killing time at 1 per cent NaOH. The data for Na<sub>3</sub>PO<sub>4</sub>. 12H<sub>2</sub>O fall along the line CJ. The equations for these lines as determined by the method of least squares are:

1.  $\log \theta = 1.7994 - 0.6121 \log C$  (10)  
(Line EG, for additions of Na<sub>2</sub>CO<sub>3</sub> to 2 per cent NaOH at 50°C.)
2.  $\log \theta = 1.6232 - 0.6541 \log C$  (11)  
(Line CH, for additions of Na<sub>2</sub>CO<sub>3</sub> to 1 per cent NaOH at 60°C.)
3.  $\log \theta = 1.6325 - 0.8308 \log C$  (12)  
(Line CJ for additions of sodium phosphate to 1 per cent NaOH at 60°C.)

In the foregoing equations " $\theta$ " is the killing time in minutes and C is

TABLE 6

EFFECT OF ADDITION OF SODIUM CARBONATE AND TRISODIUM PHOSPHATE ON GERMICIDAL EFFICIENCY OF SODIUM HYDROXIDE

% NaOH	% Na <sub>2</sub> CO <sub>3</sub>	% Na <sub>3</sub> PO <sub>4</sub> . 12 H <sub>2</sub> O	Na <sub>2</sub> CO <sub>3</sub> OR Na <sub>3</sub> PO <sub>4</sub> . 12 H <sub>2</sub> O CALC. AS NaOH in %	TOTAL CONC. OF ALKALI CALC. AS NaOH IN %	KILLING TIME IN MINUTES
Hydroxide-Carbonate at 50°C.					
2.....	0	.....	0.0	2.0	41.0
2.....	1	.....	0.76	2.76	33.8
2.....	2	.....	1.51	3.51	29.9
2.....	3	.....	2.27	4.27	25.5
Hydroxide-Carbonate at 60°C.					
1.....	0	.....	0.0	1.0	42.5
1.....	1	.....	0.76	1.76	29.0
1.....	2	.....	1.51	2.51	21.9
1.....	3	.....	2.27	3.27	20.1
Hydroxide-Phosphate at 60°C.					
1.....	.....	0	0.0	1.0	42.5
1.....	.....	1	0.32	1.32	34.9
1.....	.....	2	0.63	1.63	28.1
1.....	.....	3	0.96	1.95	24.7

the percentage concentration of alkali calculated as NaOH. The slopes of the lines EG and CH (carbonate series) are practically the same while that of CJ, for additions of phosphate, is distinctly greater than those for Na<sub>2</sub>CO<sub>3</sub>. It is evident, therefore, that trisodium phosphate is somewhat more effective than Na<sub>2</sub>CO<sub>3</sub> in producing increased germicidal efficiency of NaOH solutions. Consequently, if sodium phosphate were to be substituted for sodium carbonate in equivalent normality, the killing time equations of sodium hydroxide-sodium carbonate mixtures would indeed be very conservative estimates of the effect of sodium hydroxide-sodium phosphate mixtures.

Since the effect of adding Na<sub>2</sub>CO<sub>3</sub> to a 2 per cent NaOH solution at 50°C. is similar to adding 1 per cent sodium carbonate to a 1 per cent NaOH solution at 60°C., it is assumed that the effect of adding various amounts of carbonate to concentrations of NaOH other than 1 per cent and 2 per cent and at temperatures other than 50°C. and 60°C. will also be similar. In short,

$$\log \theta = k - d \log C \quad (13)$$

where "d" is equal to 0.6331, which is the average slope of the lines EG and CH of Figure 4;  $\theta$  is the killing time in minutes; "k" is a constant, and "C" is the percentage concentration of the total alkali (both NaOH and Na<sub>2</sub>CO<sub>3</sub>) computed as NaOH.

By means of equation (13) it is possible to calculate the killing time for various mixtures of NaOH with Na<sub>2</sub>CO<sub>3</sub> at any desired temperature, equation (4) giving the relation of killing time to concentration of NaOH alone. It should be understood that at a given temperature there is a concentration of NaOH which, when substituted in equations (4) or (13), will give the same killing times, since equation (13) is based on the total concentration of alkali which is composed of a definite percentage of NaOH to which has been added Na<sub>2</sub>CO<sub>3</sub> in concentrations from zero up.

For example, let Na<sub>2</sub>CO<sub>3</sub> be added to a 1.33 per cent NaOH solution at a temperature of 50°C. Before any carbonate is added, the killing time  $\theta_1$  may be computed from equation (4):

$$\log \theta_1 = 4.9815 - 1.7912 \log 1.33 - 0.0563 \times 50 = 1.9447 \quad (14)$$

and for mixtures of 1.33 per cent NaOH plus carbonate

$$\log \theta_1 = k_1 - 0.6331 \log 1.33 \quad (15)$$

Since equation (13) is assumed to hold for mixtures of NaOH and Na<sub>2</sub>CO<sub>3</sub>, in which the sodium carbonate concentration may increase upwards from zero,

$$"k_1" = \log \theta_1 + 0.6331 \log 1.33 = 1.9447 + 0.6331 \log 1.33 = 2.0231 \quad (16)$$

Hence, for solutions of alkali at 50°C. containing 1.33 per cent NaOH to which various amounts of Na<sub>2</sub>CO<sub>3</sub> have been added, the following equation may be used to express the relationship between concentration and killing time:

$$\log \theta = 2.0231 - 0.6331 \log C \quad (17)$$

By means of this equation (17) the killing time for a mixture of 1.33 per cent NaOH and 2.64 per cent Na<sub>2</sub>CO<sub>3</sub> has been computed to be 44.05 minutes, which agrees very well with the experimental value of 43.7 minutes. In similar manner the killing times have been computed for various mixtures of NaOH and Na<sub>2</sub>CO<sub>3</sub>, which have been employed experimentally by Levine and Buchanan,<sup>8</sup> and entered in Table 7 together with their

TABLE 7  
COMPARISON OF EXPERIMENTAL AND CALCULATED KILLING TIMES FOR DESIGNATED ALKALI MIXTURES AND TEMPERATURES

TEMP. °C.	PERCENTAGE CONCENTRATION OF ALKALI COMPUTED AS NaOH			KILLING TIME (IN MINUTES)	
	NaOH	Na <sub>2</sub> CO <sub>3</sub>	Total	Exptl.	Calc.
50	2.00	0	2.00	41.0	42.6
	2.00	0.76	2.76	33.8	33.8
	2.00	1.51	3.51	29.9	29.7
	1.33	2.64	3.97	43.7	44.1
	2.00	2.04	4.04	31.1	27.2
	2.70	1.36	4.06	18.0	19.1
	1.99	2.01	4.00	34.0*	27.1
	2.00	2.27	4.27	25.5	26.1
60	1.00	0	1.00	42.5	40.9
	1.00	0.76	1.76	29.0	28.4
	1.00	1.51	2.51	21.9	22.6
	1.00	2.27	3.27	20.1	19.1
	1.99	2.01	4.00	8.5*	7.5
70	1.00	1.00	2.00	6.3*	7.1

\*These killing times were for a commercial alkali mixture, the concentration of NaOH and carbonate of which were estimated by titration with methyl orange and phenolphthalein as indicator.

experimental values. From this table it is obvious that the computed and experimental killing times are in remarkably close agreement, indicating the applicability of equation (13).

Computation of a table of germicidal equivalents for the A.B.C.B. standard for bottle washing is complicated, as has previously been noted, by reason of the fact that a mixture of NaOH and other alkalies (3 per cent alkali of which not less than 60 per cent is caustic) is stipulated. It is therefore necessary first to determine the concentration of NaOH which has the same germicidal efficiency as a mixture of 1.8 per cent NaOH with 1.2 per cent Na<sub>2</sub>CO<sub>3</sub>. This was found to be 2.16 per cent NaOH as detailed below.

<sup>8</sup> Levine, Max, and J. H. Buchanan. Some factors influencing the germicidal efficiency of alkalies. Am. Jour. of Public Health 18: 1361-68. 1928.

TABLE 8  
GERMICAL EQUIVALENTS  
(Based on A. B. C. B. Specifications for Beverage Bottles)  
*Concentrations for Designated Temperatures and Exposure Periods\**

TIME (IN MIN.)	TEMPERATURE (°F.)													
	CONCENTRATION OF NaOH (IN %)													
	175	170	165	160	155	150	145	140	135	130	125	120	115	110
1.....	0.86	1.05	1.29	1.58	1.93	2.36	2.89	3.53	4.32	5.28	6.45	7.89	9.65	11.80
2.....	0.59	0.72	0.88	1.07	1.34	1.60	1.96	2.40	2.93	3.58	4.38	5.36	6.55	8.01
3.....	0.47	0.57	0.70	0.86	1.04	1.28	1.56	1.91	2.34	2.86	3.49	4.27	5.22	6.39
4.....	0.40	0.49	0.60	0.73	0.89	1.09	1.33	1.63	1.99	2.44	2.98	3.64	4.45	5.44
5.....	0.35	0.43	0.53	0.64	0.79	0.96	1.14	1.43	1.76	2.16	2.63	3.22	3.93	4.80
6.....	0.32	0.39	0.48	0.58	0.71	0.87	1.06	1.30	1.59	1.94	2.37	2.90	3.55	4.34
7.....	0.29	0.36	0.44	0.53	0.65	0.80	0.97	1.19	1.46	1.78	2.17	2.66	3.26	3.98
8.....	0.27	0.33	0.40	0.49	0.61	0.74	0.90	1.11	1.35	1.65	2.02	2.47	3.02	3.69
9.....	0.25	0.31	0.38	0.46	0.57	0.69	0.85	1.03	1.27	1.55	1.89	2.31	2.83	3.46
10.....	0.24	0.29	0.36	0.44	0.53	0.65	0.80	0.98	1.20	1.46	1.79	2.18	2.67	3.26
11.....	0.21	0.28	0.34	0.41	0.51	0.62	0.76	0.93	1.13	1.39	1.69	2.07	2.53	3.09
12.....	0.22	0.26	0.32	0.39	0.48	0.59	0.72	0.88	1.08	1.31	1.61	1.97	2.41	2.95
13.....	0.21	0.24	0.31	0.38	0.46	0.56	0.69	0.84	1.03	1.26	1.54	1.89	2.30	2.82
14.....	0.20	0.24	0.30	0.36	0.44	0.54	0.66	0.81	0.99	1.21	1.48	1.81	2.21	2.70
15.....	0.19	0.23	0.29	0.35	0.43	0.52	0.64	0.78	0.95	1.16	1.32	1.74	2.13	2.60

$$\log C = 2.9935 - 0.55828 \log \theta - 0.01747 t$$

$C$  = % NaOH;  $\theta$  = killing time in minutes;  $t$  = temperature in °F.

\*"Exposure Period" = "Killing Time" in experimental determinations.

From equation (4) is first computed  $\log \theta$  for a concentration of 1.8 per cent NaOH at a temperature of 54.44°C. (130°F.). This was found to be 1.4615. The value of "k" in equation (13) when "C" (concentration of caustic) is 1.8 per cent may then be determined:

$$k = \log \theta + d \log C = 1.4615 + 0.6331 \log 1.8 = 1.6209$$

The equation for determining killing time " $\theta$ " for additions of  $\text{Na}_2\text{CO}_3$  to 1.8 per cent NaOH at 54.44°C. (130°F.) thus becomes

$$\log \theta = 1.6209 - 0.6331 \log C$$

where "C" is the total concentration of alkali calculated as NaOH. For a mixture of 1.8 per cent NaOH + 1.2 per cent  $\text{Na}_2\text{CO}_3$  (i.e., 3 per cent total alkali complying with the A.B.C.D. standard) " $\theta$ " = 20.84 minutes.

The concentration of caustic alone which will kill in 20.84 minutes at 54.44°C. (130°F.) was then determined by solving for "C" in equation (4):

$$(\log 20.84 = 4.9815 - 1.7912 \log C - 0.05630 \times 54.44).$$

"C" was thus found to be 2.156 per cent NaOH.

The A.B.C.D. standard thus virtually becomes 2.16 per cent caustic for 5 minutes at 130°F. By means of the general equation

$$\log \theta = K - a \log C - bt \quad (6)$$

$K_{(A.B.C.B.)}$  may be determined, since  $\theta = 5$  minutes,  $C = 2.16$  per cent,  $t = 130^\circ\text{F}$ . are stipulated, and for disinfection with NaOH the constants  $a = 1.7912$  and  $b = 0.03129$  (when temperature is expressed in °F.) have been previously ascertained experimentally. Thus,  $K_{(A.B.C.B.)} = \log 5 + 1.7912 \log 2.16 + 0.03129 \times 130 = 5.3620$ , and the general equation

$$\log \theta = 5.3620 - 1.7912 \log C - 0.03129 t \quad (18)$$

becomes the basis for computing a table of germicidal equivalents for the A.B.C.B. standard. Since a table of equivalents in which the concentration of caustic is the variable is the most practical, equation (19), derived from equation (18)

$$\log C = 2.9935 - 0.55828 \log \theta - 0.01747 t \quad (19)$$

was employed for development of Table 8 which shows the concentration of NaOH at a stipulated temperature and time which will effect the sterilizing action equivalent to that of 2.16 per cent NaOH at 130°F. for 5 minutes.

If temperature is expressed in °C., equation (18) for the A.B.C.B. standard becomes

$$\log \theta = 4.3596 - 1.7912 \log C - 0.05630 t \quad (20)$$

#### COMPARISON OF BOTTLE WASHING STANDARDS

The foregoing equations for determination of germicidal equivalents for a stipulated standard may be readily employed for comparing different

standards, promulgated for bottle washing, with one another. This might be done by comparing the values of "K" in the respective equations, or any one of the three factors—killing time, temperature, or concentration of NaOH—in the various tables of germicidal equivalents, when the other two factors are constant, as may be seen from the following examples:

Suppose that two standards, A and B are considered. Their respective equations would be

$$(A) \log \theta_A = K_A - a \log C_A - bt_A$$

$$(B) \log \theta_B = K_B - a \log C_B - bt_B$$

$K_A$  and  $K_B$  being constants characteristic of the resistance of the hypothetical organisms formulated, as previously explained, by the respective standards; (the constants "a" and "b", it should be recalled, are characteristic for the germicide and independent of the test organism). For a stipulated temperature and concentration it is evident that "K" is a function of the logarithm of the killing time  $\theta$ .

Now, subtracting equation (B) from (A) there is obtained

$$\log \theta_A - \log \theta_B = K_A - K_B - a \log C_A + a \log C_B - bt_A + bt_B \quad (21)$$

and when  $C_A = C_B$  and  $t_A = t_B$ , which would be the case if killing times for the two standards under consideration were being determined for a stipulated concentration of NaOH and temperature, equation (21) becomes:

$$\log \theta_A - \log \theta_B = K_A - K_B$$

$$\log \frac{\theta_A}{\theta_B} = K_A - K_B.$$

Thus, the difference in the values for "K" in the equations for germicidal equivalents of any two standards is the logarithm of the ratio of the killing times at any given concentration of NaOH and temperature for the hypothetical organisms formulated by the respective standards.

The values determined for "K" (temperature expressed in °F.) were found to be as follows:

New York City .....	$K = 6.0777$
A. B. C. B. .....	$K = 5.3620$
California .....	$K = 5.1670$
Chicago .....	$K = 4.8195$

The New York City and Chicago standards are both for milk bottles. The difference in their "K" values is 1.2582, the anti-log of which is 18.1; thus, for any given temperature and concentration it would be necessary to expose a bottle 18.1 times as long to meet the New York City as would be required to comply with the Chicago requirements.

The A. B. C. B. standard is not directly comparable with the above since it is designed for a different purpose, namely for carbonated beverage containers. Merely for illustrative purposes, however, it may be

pointed out that on the above basis it would be necessary to expose a milk bottle about 5 times as long to meet the new York City standard but only about one-third as long to meet the Chicago standard as is required by the A.B.C.B. for a beverage bottle at any stipulated temperature and concentration of NaOH.

Tables of equivalent germicidal efficiency, such as Tables 3, 4, 5, and 8, may be readily employed for comparing standards; thus, at a temperature of 130°F. for 5 minutes, a concentration of 1.05 per cent NaOH is required on the basis of the Chicago standard (Table 4), whereas from Table 3 it is noted that 5.4 per cent caustic would be necessary to comply with the New York City standard. Similarly, it will be noted that for any stipulated temperature and killing time the ratio of caustic required to meet the respective standards is approximately 5 to 1.

A more general view of the relation between bottle washing standards, in respect to the concentration of caustic required at stipulated temperatures and exposure periods, may be obtained from a consideration of the equations from which Tables 3, 4, 5, and 8 were computed. These are recapitulated herewith:

$$\log C_{(N.Y.C.)} = 3.3931 - 0.55828 \log \theta - 0.01747 t \quad (9a)$$

$$\log C_{(A.B.C.B.)} = 2.9935 - 0.55828 \log \theta - 0.01747 t \quad (19)$$

$$\log C_{(California)} = 2.8843 - 0.55828 \log \theta - 0.01747 t \quad (9c)$$

$$\log C_{(Chicago)} = 2.6906 - 0.55828 \log \theta - 0.01747 t \quad (9b)$$

Since the coefficients of "θ" and "t" are the same in each of the equations, then, for a stipulated temperature and exposure period, (i.e., killing time) the difference between equations 9a and 9b for example, becomes

$$\log C_{(N.Y.C.)} - \log C_{(Chicago)} = 3.3931 - 2.6906 = 0.7025,$$

the antilog of which is 5.0; that is, for a given temperature and exposure period, 5.0 times the concentration of caustic is required by New York as by Chicago. Similarly, it will be found that the New York City standard for milk bottles requires 3.2 times as much caustic as is stipulated by California and 2.5 times the concentration recommended by the A.B.C.B. for beverage bottles.

#### SUMMARY

Mathematical derivation of equations for ascertaining relationship between temperature, concentration of caustic solution, and period of exposure, to effect germicidal actions equivalent to those stipulated in the respective standards for bottle washing by the food control authorities in New York, Chicago, California, and by the American Bottlers of Carbonated Beverages are detailed.

The general equation for the period of exposure (or killing time) is

$$\log \theta = K - a \log C - bt$$

where "θ" is time in minutes, "C" is concentration of NaOH expressed

in percentage, "t" is temperature, "a" and "b" are coefficients determined experimentally and constant for a given germicide, and "K" is a constant characteristic of the resistance of a hypothetical organism stipulated by a given standard. For NaOH as the germicide, "a" was found to be 1.79120, and "b" is 0.05630 when temperature is expressed in °C. and 0.03129 if temperature is expressed in °F. The values for "K" were as follows:

Standard	Temp. in °C.	Temp. in °F.
New York .....	5.0753	6.0777
A.B.C.B. .....	4.3596	5.3620
California .....	4.1646	5.1670
Chicago .....	3.8171	4.8195

Tables showing the concentration of NaOH required to produce an equivalent germicidal effect for a stipulated temperature and period of exposure (killing time) are presented for each of the standards considered. The equations employed were derived from those for "killing time," the general equation being

$$\text{Log } C = k' - 0.55828 \log \theta - 0.01747 t$$

when "C" is concentration of NaOH in percentage; "θ" is the period of exposure or "killing time" in minutes; "t" is temperature in °F., and "k'", which is a constant characteristic for any stipulated standard under consideration, was found to be as follows:

New York .....	3.3931
A.B.C.B. .....	2.9935
California .....	2.8843
Chicago .....	2.6906

The differences in the "K" values of any two standards is the logarithm of the ratio of their respective "killing times" (for a stipulated temperature and concentration of NaOH), while the difference in the "k" values is the logarithm of the ratio of the respective concentrations of NaOH (for a given temperature and killing time) which will produce equivalent germicidal effects.

# TICKS COLLECTED ON THE TAMA (IOWA) INDIAN RESERVATION WITH NOTES ON OTHER SPECIES<sup>1</sup>

GAINES W. EDY AND CHAS. R. JOYCE

From the Section of Entomology and Economic Zoology, Iowa Agricultural Experiment Station

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Biological studies on the American dog tick, *Dermacentor variabilis*, were begun in April, 1941, on the Tama Indian Reservation, Tama, Iowa. The work is being conducted jointly by the Iowa State Board of Health and the Department of Zoology and Entomology, Iowa State College. The authors are greatly indebted to Dr. W. L. Bierring and Dr. Carl F. Jordan of the Iowa State Board of Health for financial support and assistance in the work; to Dr. F. C. Bishopp, Bureau of Entomology and Plant Quarantine, U. S. Department of Agriculture, for tick records in Iowa; to Dr. R. A. Cooley, U. S. Public Health Service, Rocky Mountain Laboratory, for the determination of several species; and to Dr. C. J. Drake and Dr. E. R. Becker of Iowa State College for advice and assistance in the work.

This paper is a report concerning the ticks collected on the Reservation and other species known for Iowa. There has been no systematic survey of the ticks in the state, so it is not thought that the species herein listed represent a complete list for Iowa. Six of the fourteen species given below, *Dermacentor variabilis*, *Haemaphysalis leporis-palustris*, *Ixodes sculptus*, *I. texanus*, *Amblyomma americanum*, and *A. ovale*, were collected on the Tama Indian Reservation; records of five species were taken from the literature; two were from collections of individuals; and one was from the Accession Catalogue of the Division of Insects Affecting Man and Animals, Bureau of Entomology and Plant Quarantine, U. S. Department of Agriculture. However, *D. variabilis*, *H. leporis-palustris*, *A. americanum*, *I. sculptus*, *I. cookei*, and *I. kingi* are also listed in the Accession Catalogue from Iowa.

## FAMILY ARGASIDAE CANESTRINI

The family Argasidae is represented in Iowa by three genera and three species, *Argas persicus*, *Ornithodoros kelleyi*, and *Otobius megnini*. *O. kelleyi* may occur here naturally, but the other two species are southern forms.

### *Argas persicus* (Oken)

The normal range of this species lies in the southwestern part of the United States, California, Arizona, New Mexico, Texas, and Florida. The Marx Collection of the National Museum contains specimens from Iowa, but these probably represent the only record of its presence in the state.

<sup>1</sup>Journal Paper No. J-791 of the Iowa Agricultural Experiment Station, Ames, Iowa. Project No. 372.

The tick may have accompanied a shipment of chickens, which could have easily been true of the larval form, since several days are required for engorgement. It is an important parasite of poultry in the southern states and a vector of fowl spirochaetosis.

*Ornithodoros kelleyi* Cooley and Khols.

The description of *O. kelleyi* appeared in print in April, 1941. The type is a nymph collected in Utah. A single female specimen collected in a dwelling house at Dubuque, Iowa, in March, 1939, was recently given to the senior author by Dr. E. R. Becker of Iowa State College. According to Dr. R. A. Cooley, this tick has an eastern range and is likely a parasite of bats. There are probably other members of this genus present in Iowa that have not as yet been collected.

*Otobius megnini* (Duges)

According to Banks (1908), *O. megnini* has been collected in Iowa at Ames and Davenport. Specimens are represented in the collection of the Museum of Comparative Zoology, Cambridge, Massachusetts, and in the Marx Collection of the National Museum.

This tick is most prevalent in the semiarid sections of southwestern United States where it is an important parasite of livestock. The immature forms remain attached in the ears of its host for several months, and the shipment of infested stock to the more northern states accounts for its presence there.

FAMILY IXODIDAE MURRAY

Five genera and eleven species make up the known ticks of this family in Iowa. The genus *Ixodes* is the largest and, taxonomically, the most difficult group of ticks in America. It is not, however, as important from a medical standpoint as some of the other genera. A number of the species have widely scattered distribution and feed on smaller rodents. Life histories have not been worked out for the majority of the species and their taxonomic variations are not well known.

*Ixodes angustus* Neumann

The type was collected in Idaho from the bushy-tailed wood rat (*Neotoma cinerea occidentalis*). It has a range in the northern part of the United States and is very common in British Columbia. According to Hooker (1909), there are specimens from Iowa in the collection of the Bureau of Animal Industry.

*Ixodes scapularis* Say

Banks (1908) states that this tick was taken at McGregor, Iowa. Hooker (1909) mentions its presence in the Museum of Comparative Zoology. Its natural range is southern and southeastern United States. Both large and small animals serve as hosts. Deer are often heavily parasitized during the fall and early winter in Oklahoma. It is a known vector of anaplasmosis.

*Ixodes cookei* Pack.

This tick has a rather wide distribution in the United States. We have specimens from Clayton and Blackhawk Counties. The Accession Catalogue of the Division of Insects Affecting Man and Animals lists a collection from Clarke County.

Hosts include a large number of the smaller mammals. The wood-chuck is a very common host in the United States and British Columbia.

*Ixodes sculptus* Neumann

The distribution of this tick is not so well known as the above species, but apparently it is a rather widely scattered species. The life history was worked out by Hixon (1932).

We have specimens from Tama and Story Counties in Iowa. It also occurs in Shelby, Clarke, and Cerro Gordo Counties, according to the records of the Division of Insects Affecting Man and Animals.

The smaller animals, especially the ground squirrel group, serve as principal hosts. All stages (except the male) may be found on the same animal. The thirteen-striped ground squirrel was found heavily infested several times during the summer on the Tama Indian Reservation.

*Ixodes kingi* Bishop

The rotund tick is likely an uncommon species here. We know of only two records and these are contained in the files of the Bureau of Entomology. One specimen was taken in Cerro Gordo County and another in Plymouth County. This tick has a western range and is common in the Rocky Mountains. It has been found attached to a variety of animals.

*Ixodes texanus* Banks

This tick is apparently represented in Iowa by a single collection made by the authors. A female was removed from a dog on May 1, 1941, at Tama, Tama County, Iowa.

According to Hearle (1938), it is common in certain areas of British Columbia. It is mainly a parasite of rodents.

*Rhipicephalus sanguineus* Latreille

The brown dog tick is one of the most widespread species of ticks. It was formerly known only from Texas and Florida, but has now been collected in some twenty-five scattered states. It is represented in Iowa by a single collection. A few larval specimens were sent to the Department of Zoology and Entomology, Iowa State College, in August, 1940, by W. A. Hauber, St. Ambrose College, Davenport. The dog is the principal host and acts as the chief agent in its dissemination.

*Haemaphysalis leporis-palustris* Pack

The rabbit tick is one of the commonest and most widespread species in North America and no doubt occurs throughout the entire United States. We have specimens from Boone, Story, and Tama Counties.

Rabbits and hares are the main hosts for the adult stage. A number of smaller animals and a long list of birds serve as hosts for the immature forms.

#### *Amblyomma americanus* Linnaeus

The lone star tick has a natural range in the southeastern United States, but has been collected as far north as Labrador and as far south as Brazil. It is apparently established in Iowa, but is not common. The tick was collected several times during the past summer on the Indian Reservation; it has also been taken in Clarke County.

It attacks a large number of animals, and all stages are known to attack man. Experimentally it has been shown to transmit spotted fever and tularemia.

#### *Amblyomma ovale* Koch

According to Nuttall and Robinson (1926), this tick occurs in Central and South America, from Mexico to the Argentine Republic. A single male was removed from a dog in September, 1941, on the Indian Reservation, Tama County. This same dog had been examined only four days previously and, apparently, had not been off the Reservation during the entire summer. The Indians held their annual Pow-wow during August, and several persons attended the celebration from some of the more southern states. This is not, however, offered as an explanation for its presence on the Reservation. We were unable to obtain any definite or positive information relative to its occurrence in Iowa. The specimen was kept alive for several days in the laboratory.

#### *Dermacentor variabilis* Say.

The American dog tick is distributed throughout the eastern half of the United States and on the Pacific coast. It is probably present over most of the state. Specimens have been collected by the authors in Clarke, Story, and Tama Counties. The southern part of the state is well represented by this tick, according to the records of the Iowa State Board of Health.

The dog serves as the principal host for the adult stage. The tick is known to attack a large number of animals, including man. Mice and rats appear to be the most important hosts for the immature forms. The cottontail rabbit may also be an important host, especially during the spring period. *D. variabilis* is a known vector of spotted fever, tularemia, and anaplasmosis.

#### SUMMARY

The family *Argasidae* is represented in Iowa by *Argas persicus*, *Otobius megnini*, and *Ornithodoros kelleyi*. The latter species may have a natural range in this state, but the first two do not commonly occur this far north. So far as is known, they do not attack man in this country.

The family *Ixodidae* is represented by five genera and eleven species; the genus *Ixodes* by *angustus*, *scapularis*, *cookei*, *sculptus*, *kingi*, and *ter-*

*anus; Rhipicephalus by sanguineus; Haemaphysalis by leporis-palustris; Amblyomma by americanum and ovale; and Dermacentor by variabilis.* Six species, *Dermacentor variabilis*, *Ixodes sculptus*, *I. texanus*, *Haemaphysalis leporis-palustris*, *Amblyomma americanum*, and *A. ovale*, were collected on the Tama Indian Reservation.

*Dermacentor variabilis* and *Amblyomma americanum* are the only two ticks now known to occur in Iowa that are considered important parasites of man. The people are concerned largely with the former species, since *A. americanum* is not common in the state. Probably more than 99 per cent of the tick bites on humans can be attributed to *D. variabilis*.

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